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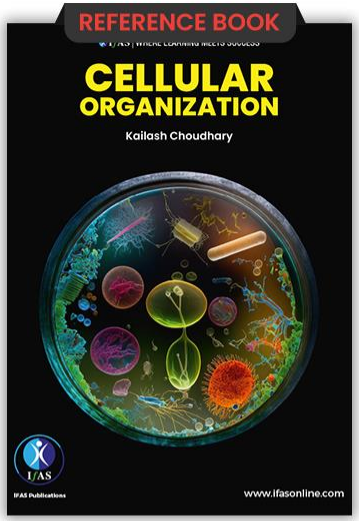
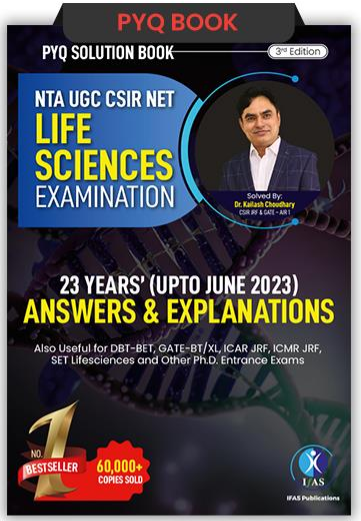
CSIR NET – Life Science

Unit 2: Cellular Organization

L13 Cytoskeleton and cell wall

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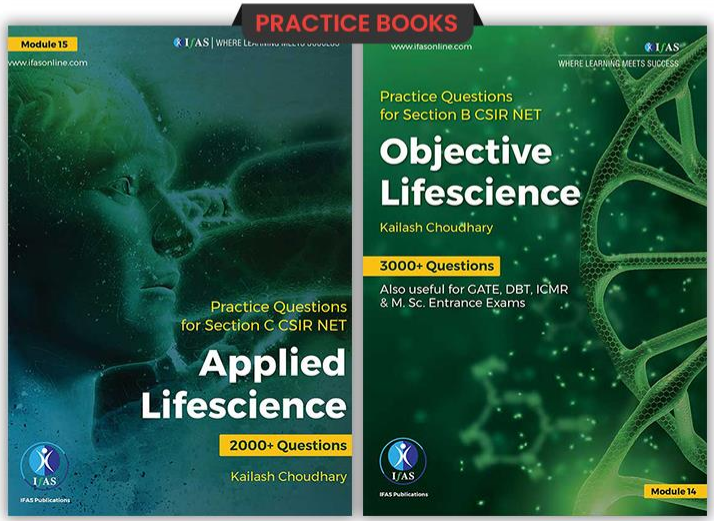

IfAS

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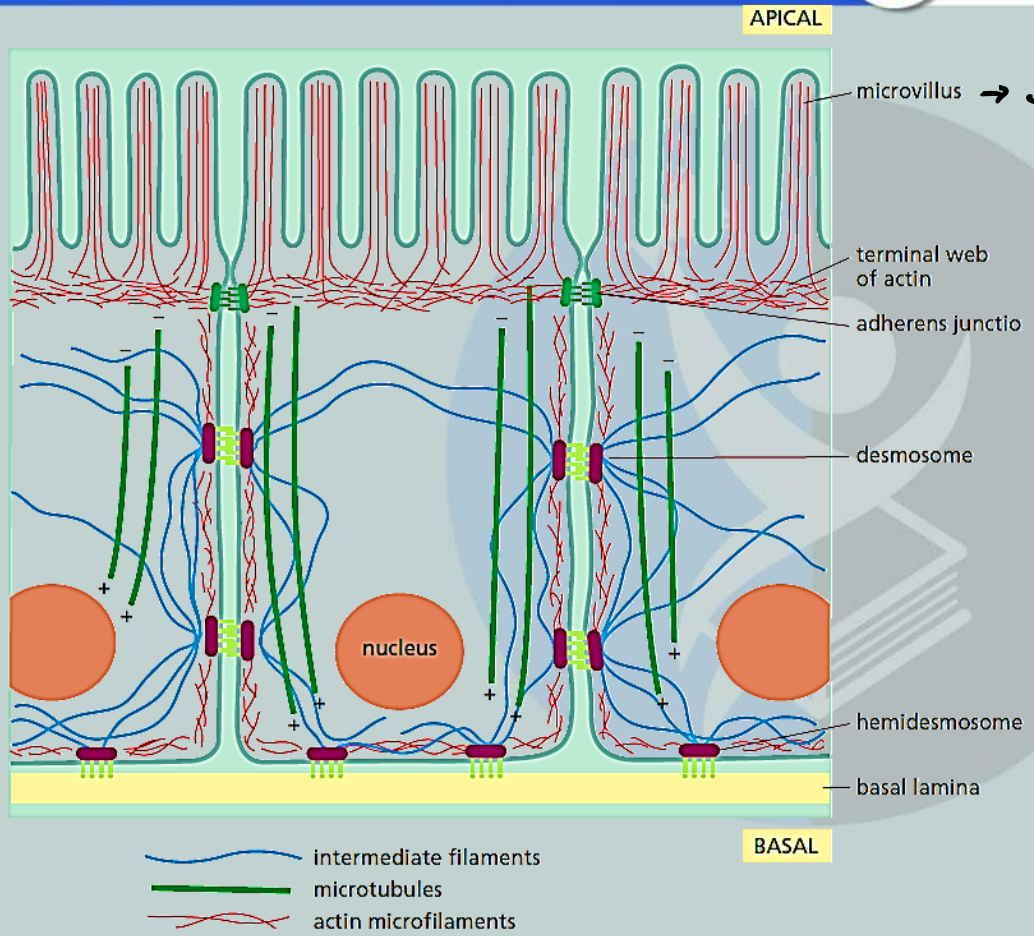
Points to be covered in this Lecture

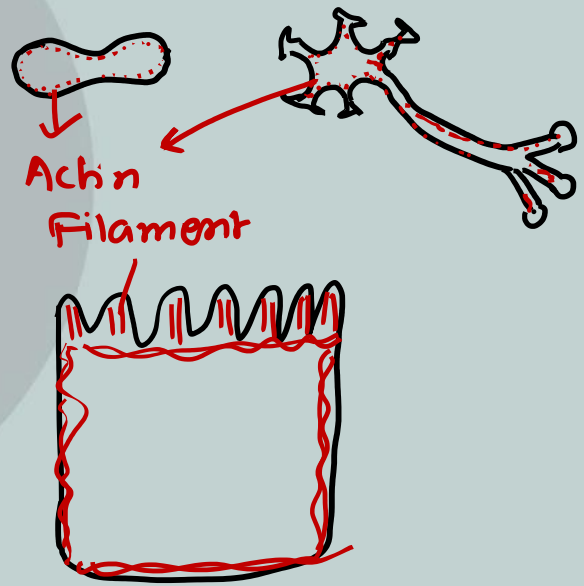
- **Functions of actin**
- Microtubule and Molecular motors
- Intermediate filament
- Cell wall

Functions of actin filament

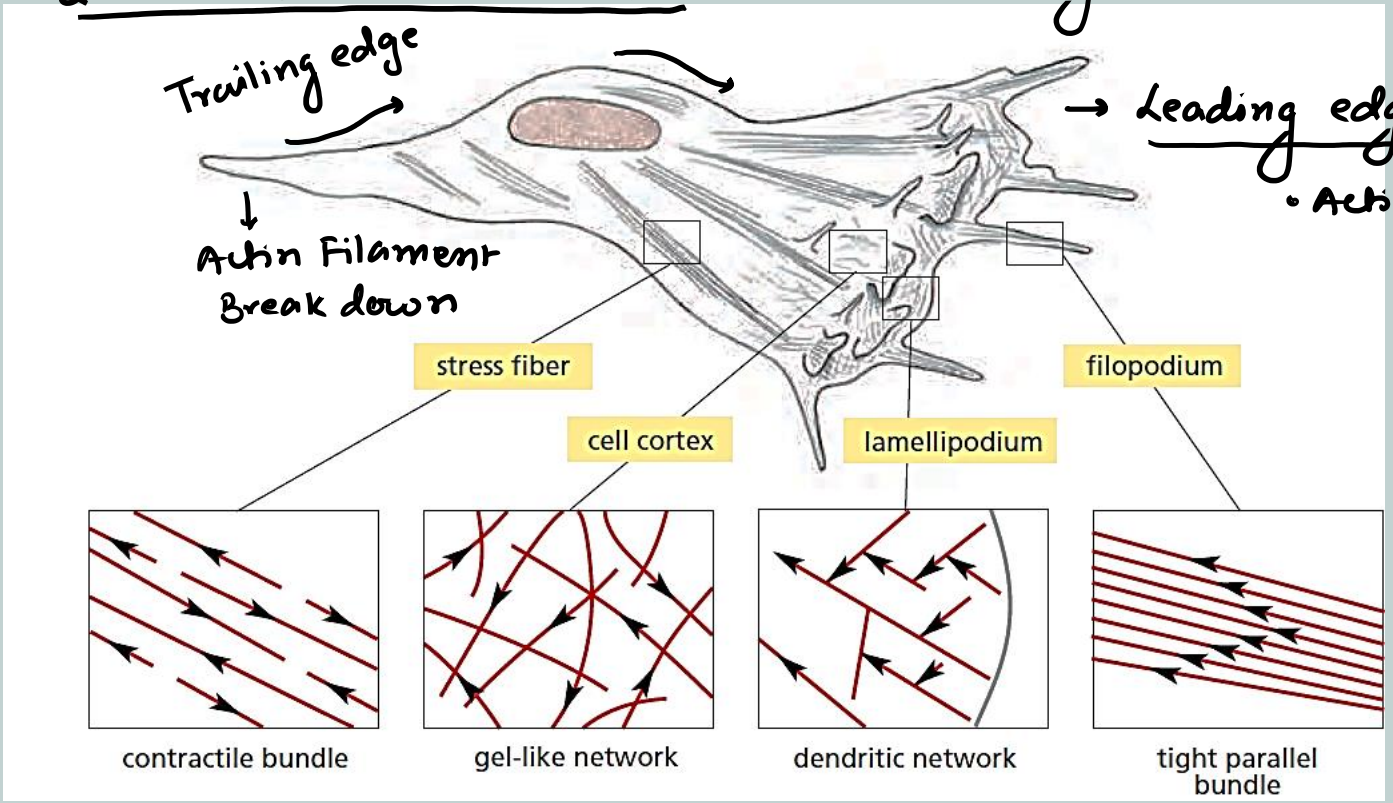
1. Helps in cell shape maintenance ✓ and also form small projections of the cell
2. Cellular movement ✓

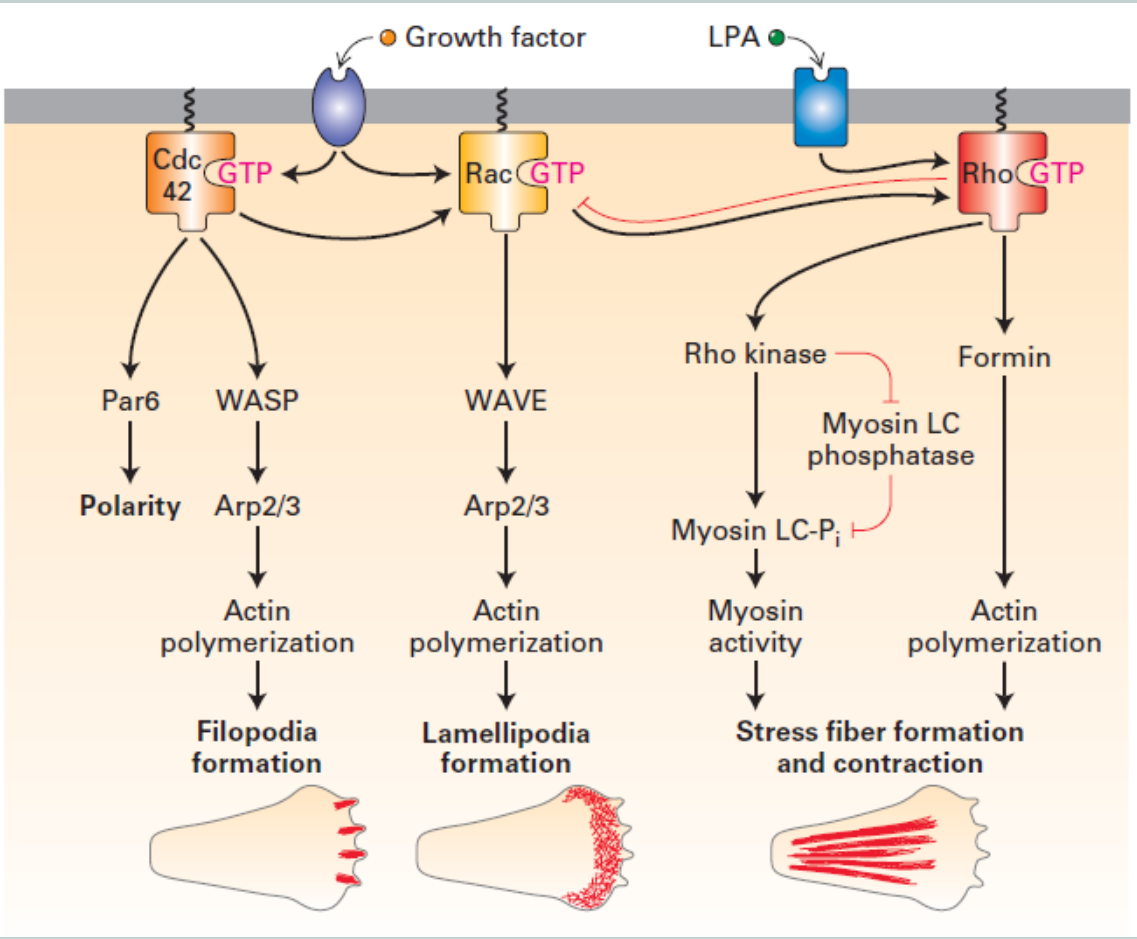


microvillus → Small Projection (By actin)



2. Cellular movement → Actin Rearrangement





CDC-42-GTP	Filopodia Lamellipodia
RAC-GTP	Lamellipodia
Rho-GTP	Stress Fiber

Bacteria lack true cytoskeletal systems but have structurally similar components to those in eukaryotes

- the **actin-like** *MreB* protein is involved in DNA segregation,
- the **tubulin-like** *FtsZ* protein is involved in determining where bacterial cells will divide, and
- the **intermediate filament**-like *crescentin* protein is an important regulator of cell shape.

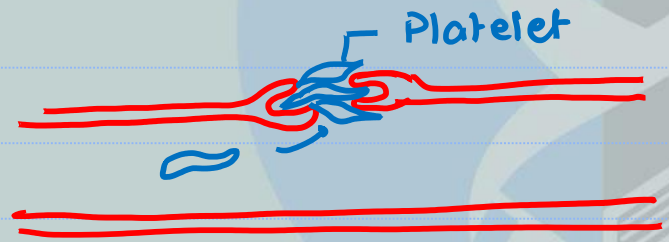
Actin also play role in platelet plug formation

↓

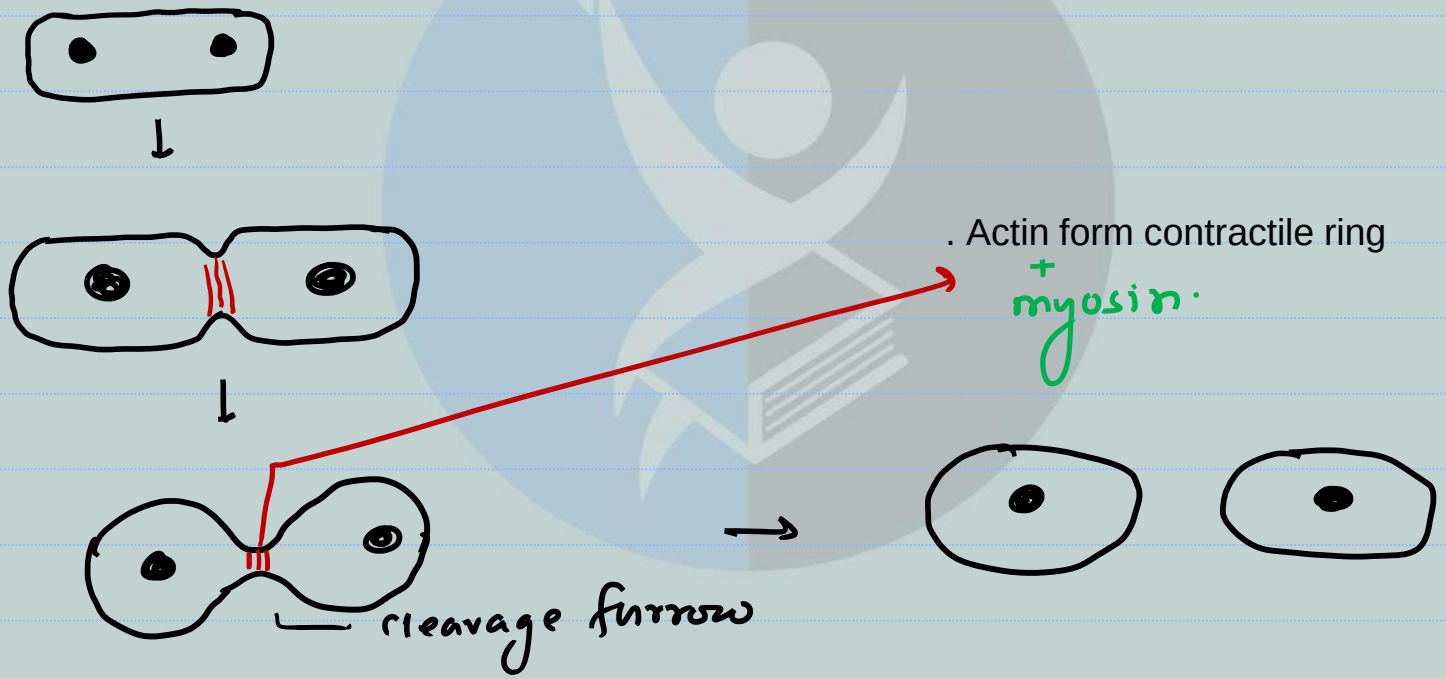
Platelet attached damaged endothelial cell block the damage gap

↓

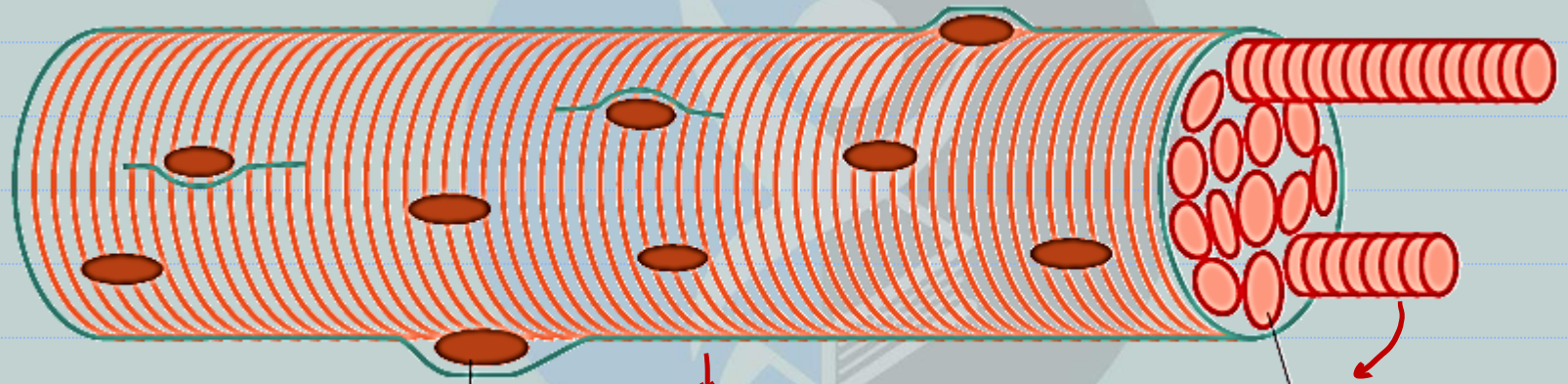
Platelet shape change due to actin rearrangement



Actin also involve in cytokinesis



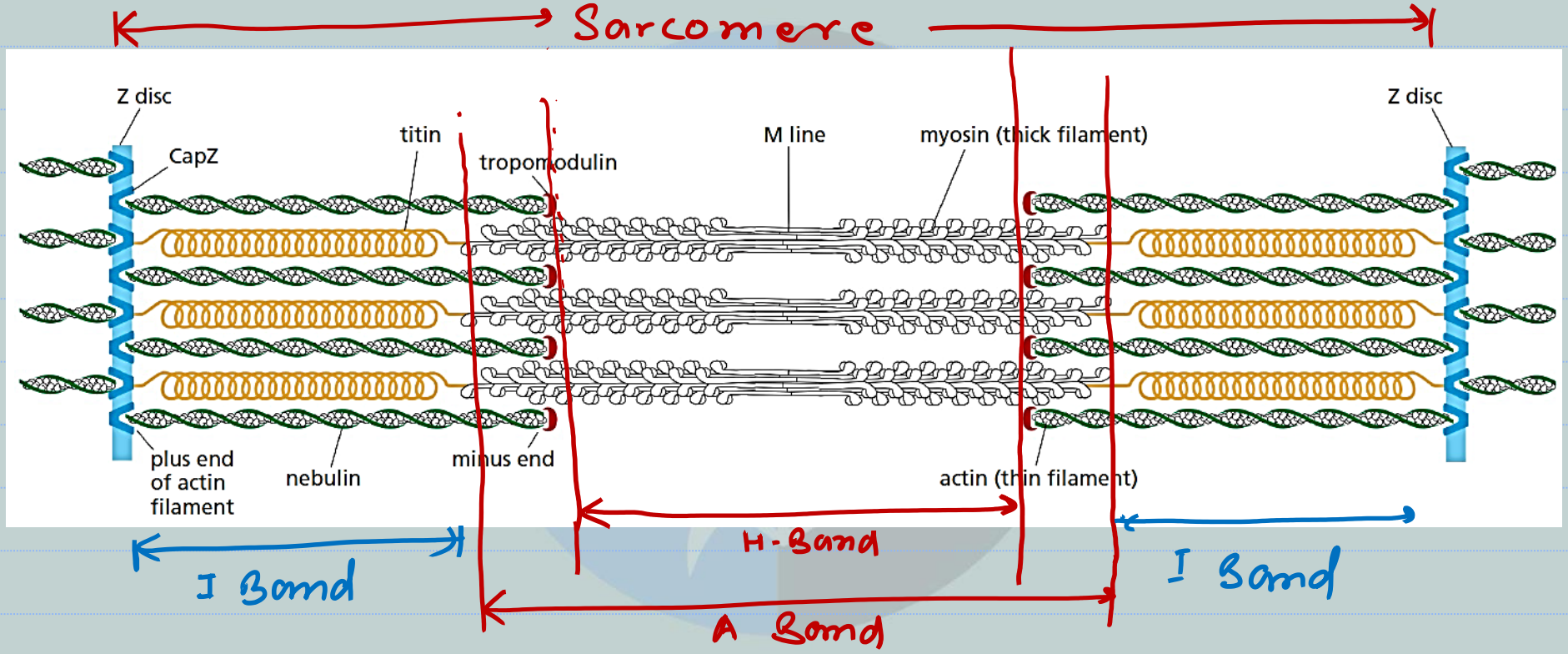
Actin myosin play role in muscle contraction



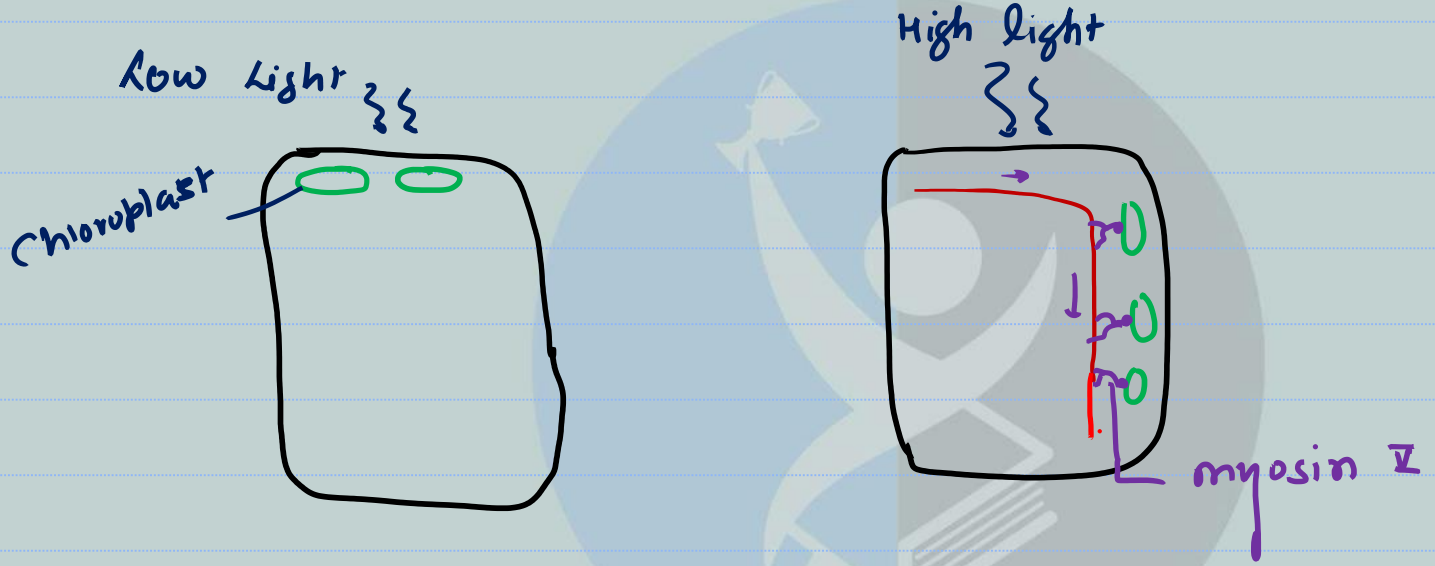
- Contain Sarcomere
  - Actin + myosin.

During muscle Contraction

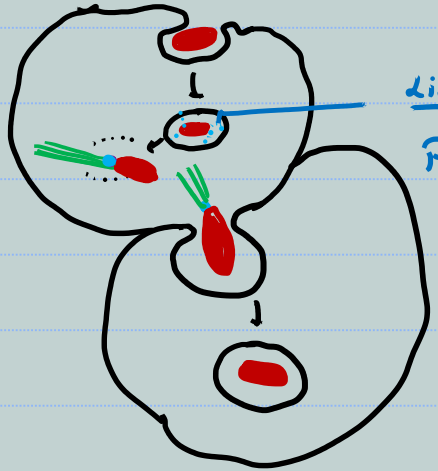
- Due to myosin motor activity
  ↓
  Sarcomere length ↓se
  - H Band length = ↓
  - I Band length = ↓se
  - A Band length - remain same.


Sarcomere
Z disc
CapZ
titin
tropomodulin
M line
myosin (thick filament)
Z disc
plus end of actin filament
nebulin
minus end
actin (thin filament)
H-Band
I Band
I Band
A Band

Movement of chloroplast

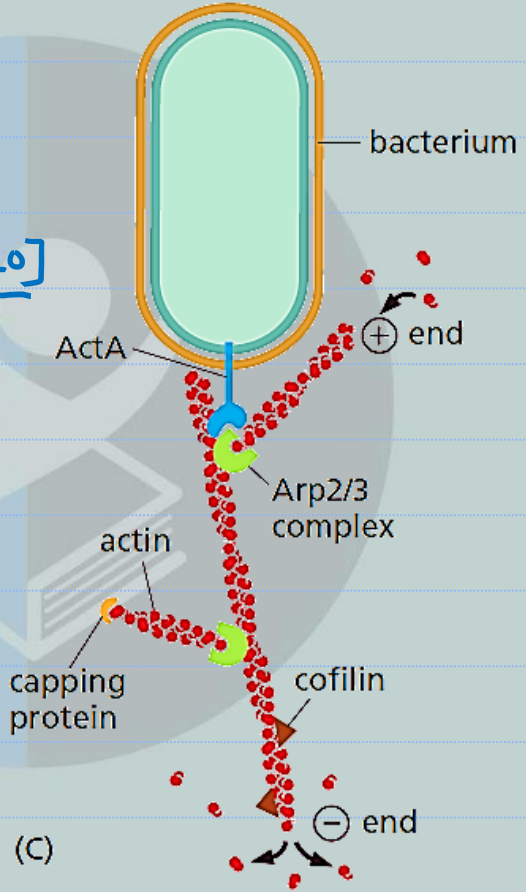


Intracellular movement of Listeria monocytogenes



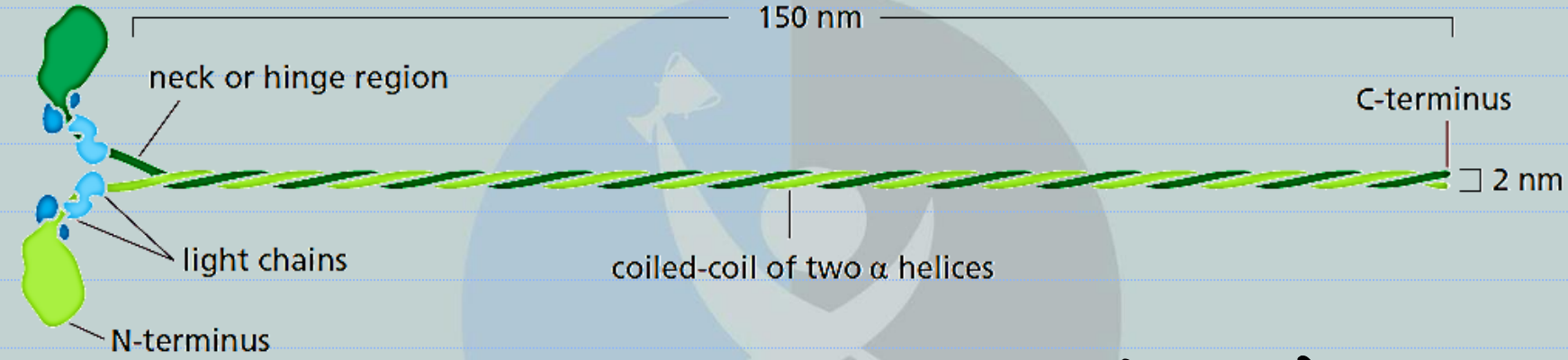
listeria lysin - O [LLO]

Phagosome disrupt



Actin involve in acrosomal extension

- occurs during fertilization (in sperm)

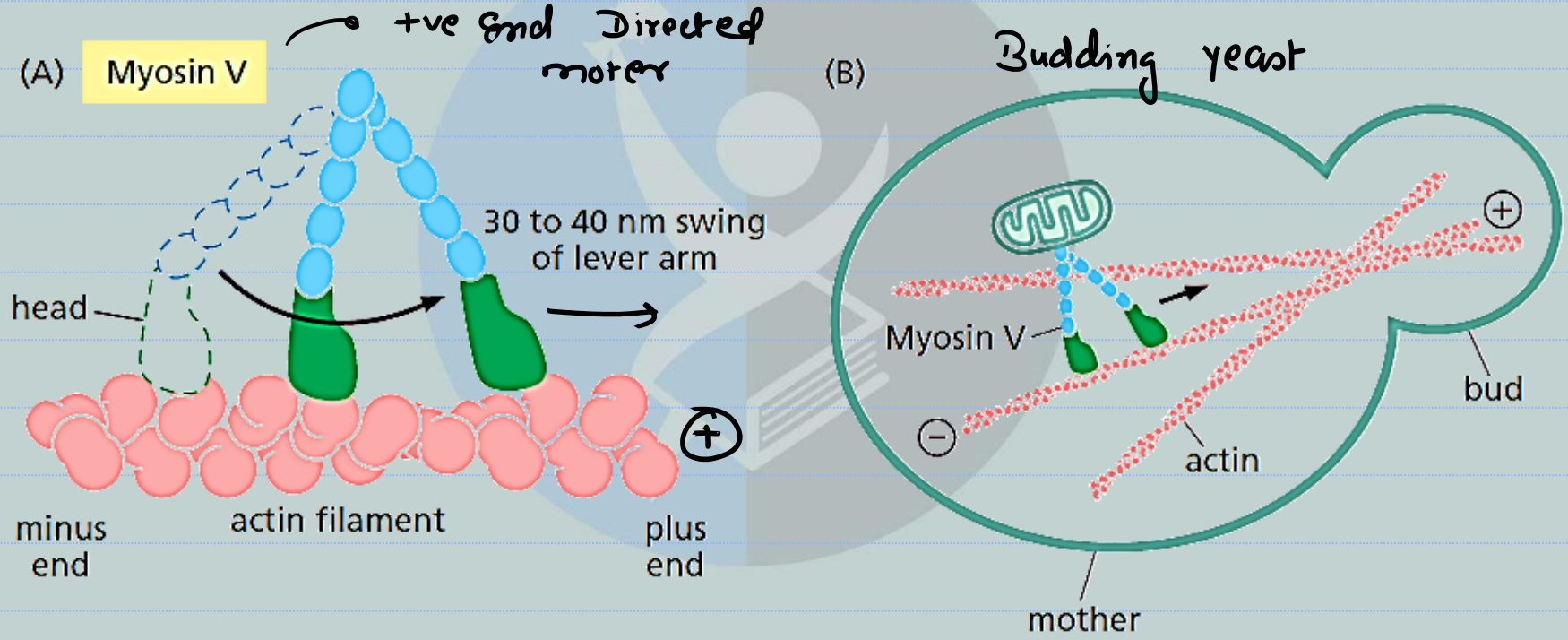


myosin – II motor → involve in muscle Contraction

↓

(+ve end directed)

Organelle movement

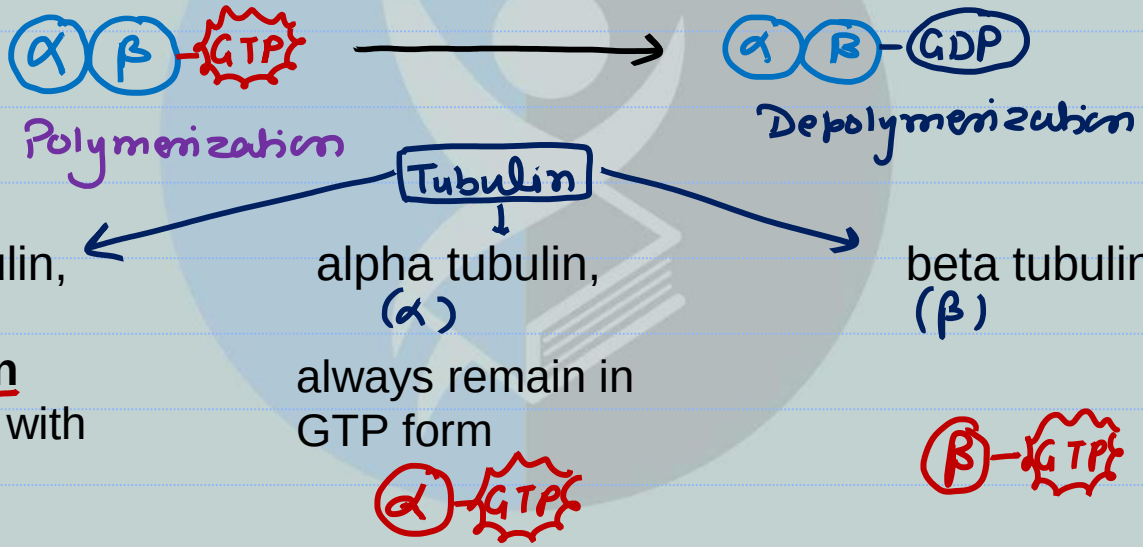


Drug that inhibit polymerization of actin

- **Cytokinesin** D inhibits polymerization.
  (Fungal toxin.)
- **Latriculin** A inhibits polymerization of actin (Binds with G-actin ADP and prevent its conversion into G-actin ATP).
- **Phalloidin** inhibits depolymerization of actin. (Stabilize actin filament)

Microtubule

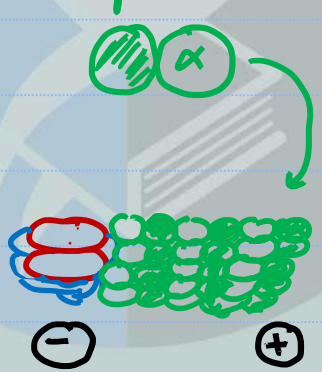
Microtubule made up of tubulin heterodimer



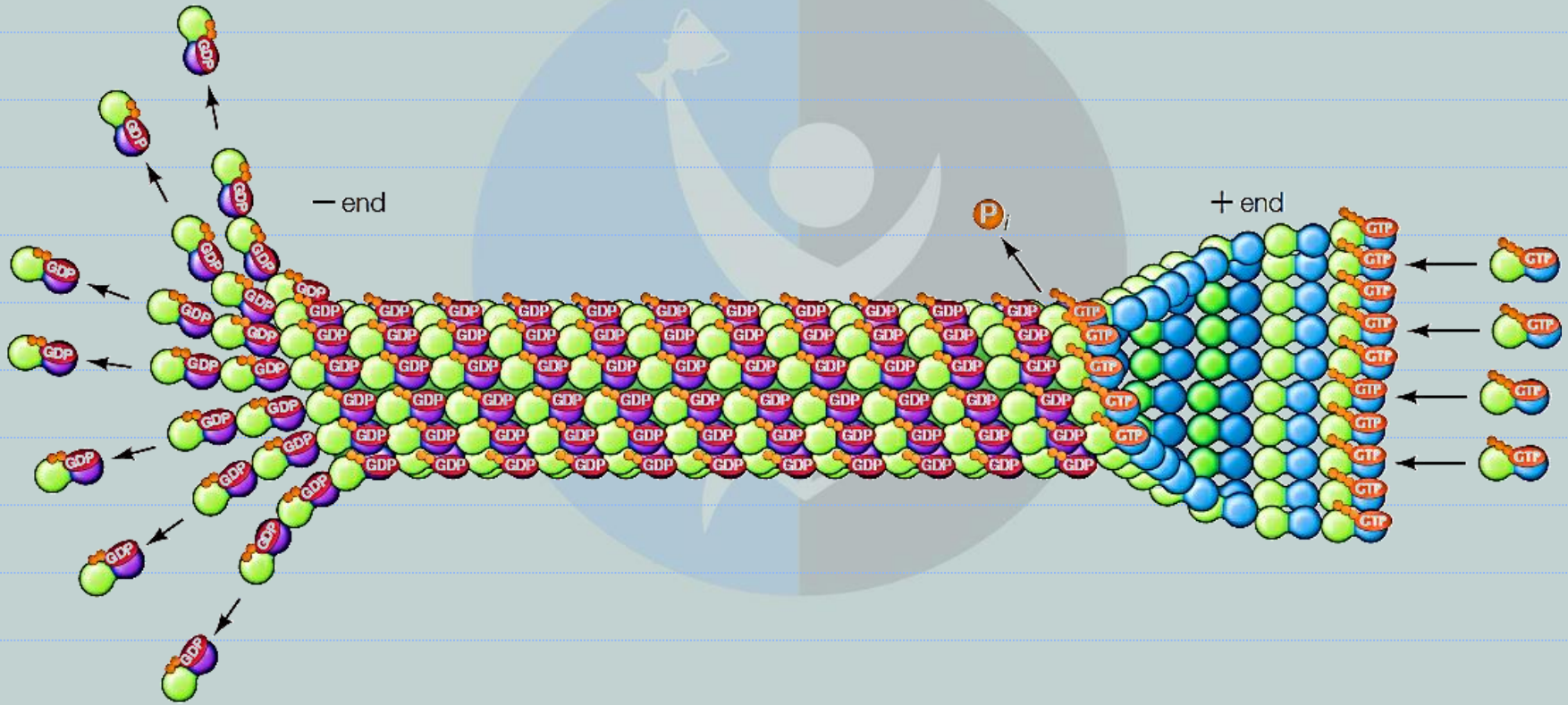
Microtubule filament formation - 3 steps -

1. Nucleation,
2. Elongation,
3. Dynamic instability

γ - Tubulin
+
other proteins

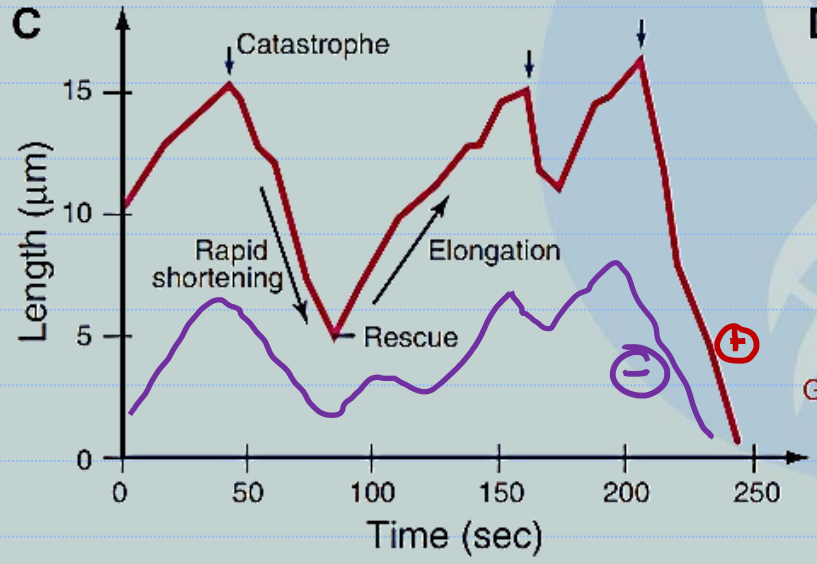


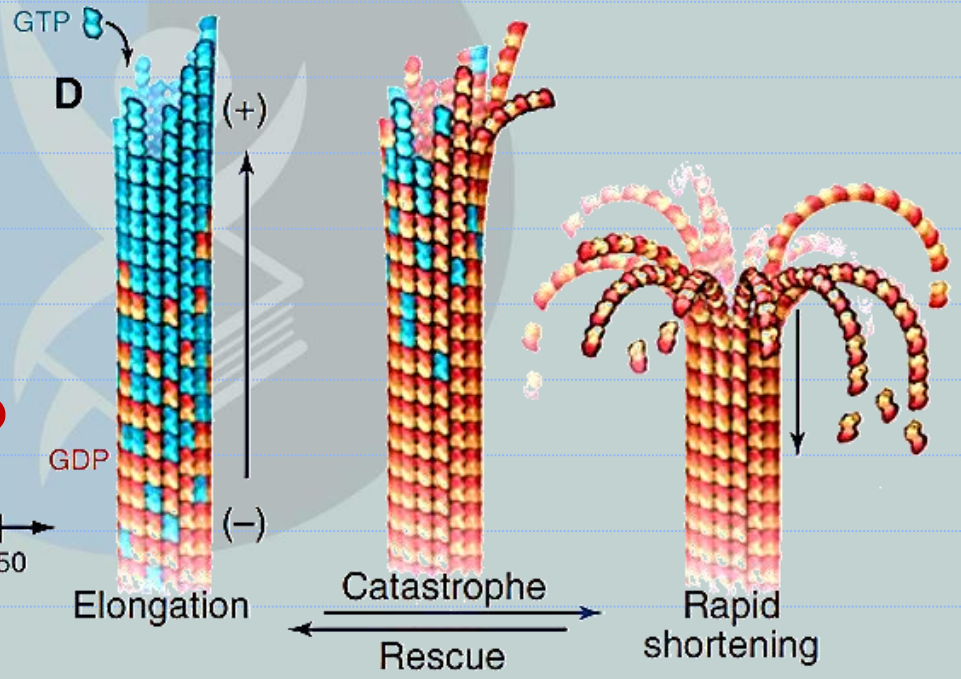
Elongation



Dynamic instability

Dynamic instability Rapid polymerization followed by rapid depolymerization



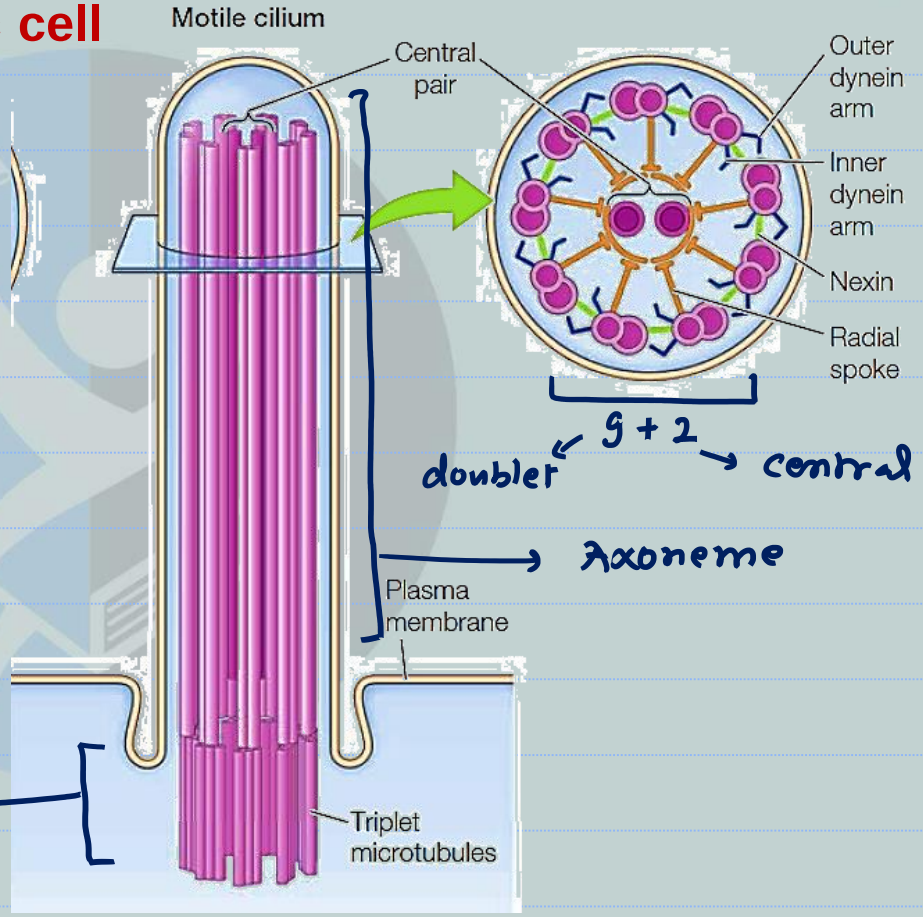


Microtubule present in eukaryotic cell

Formation of cilia and flagella

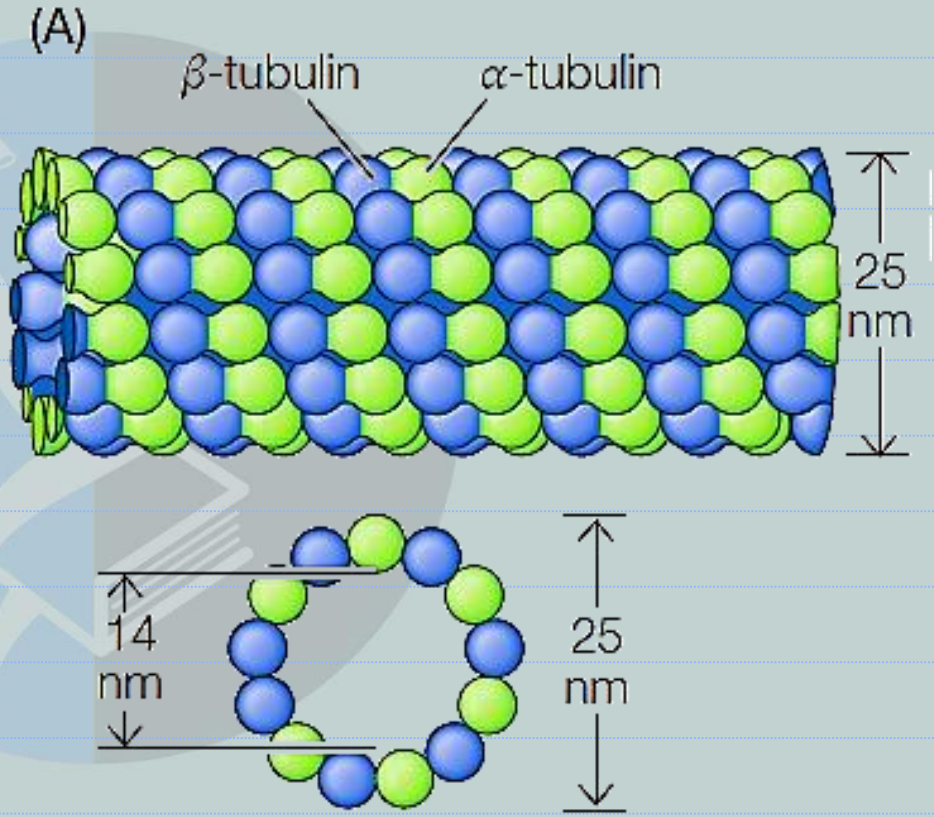
↓

long projections

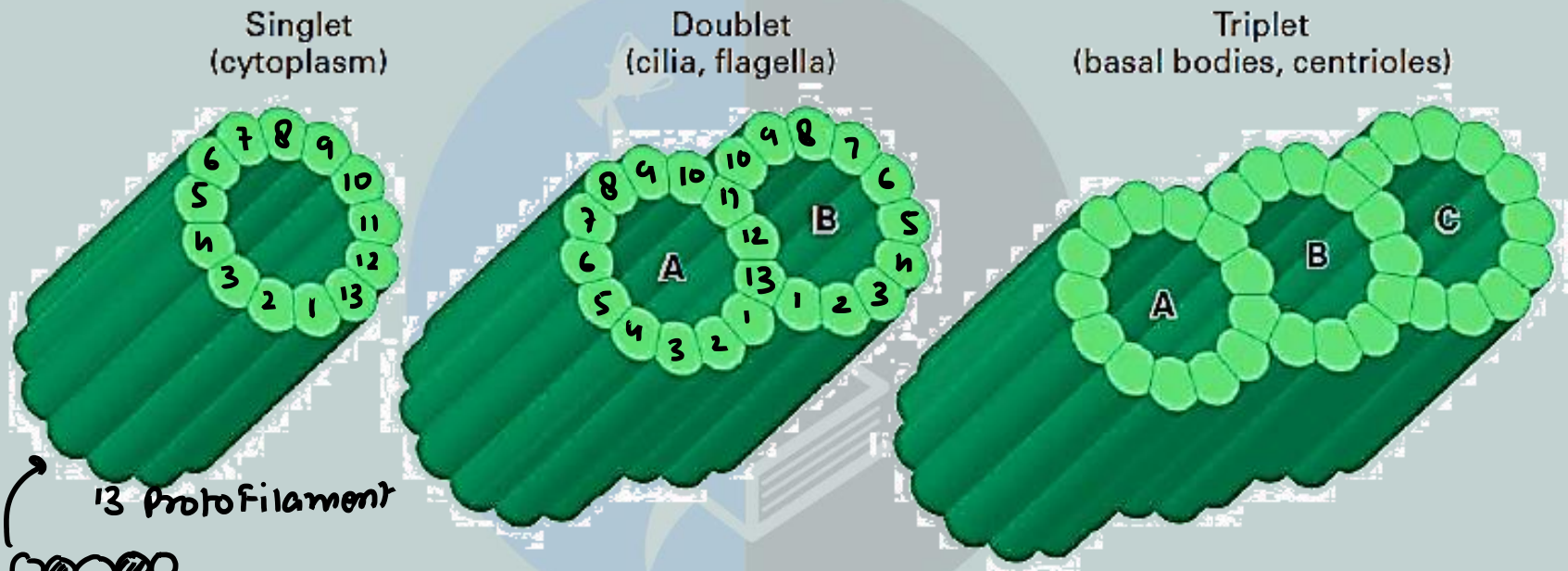


[9 + 0 ↓ Tripler] ← Basal Body ←

13 protofilament form the wall of microtubule



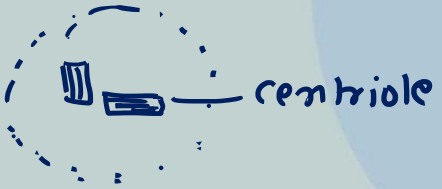
Gmp


Singlet
(cytoplasm)
Doublet
(cilia, flagella)
Triplet
(basal bodies, centrioles)
A
B
A
B
C
13 Protofilament
1
Protofilament

Microtubule organizer reason

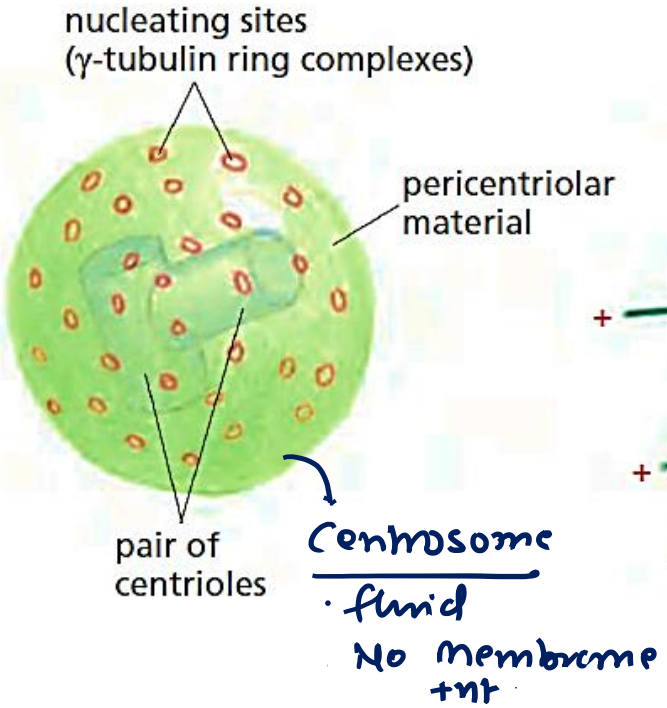
Centrosome (9 + 0)

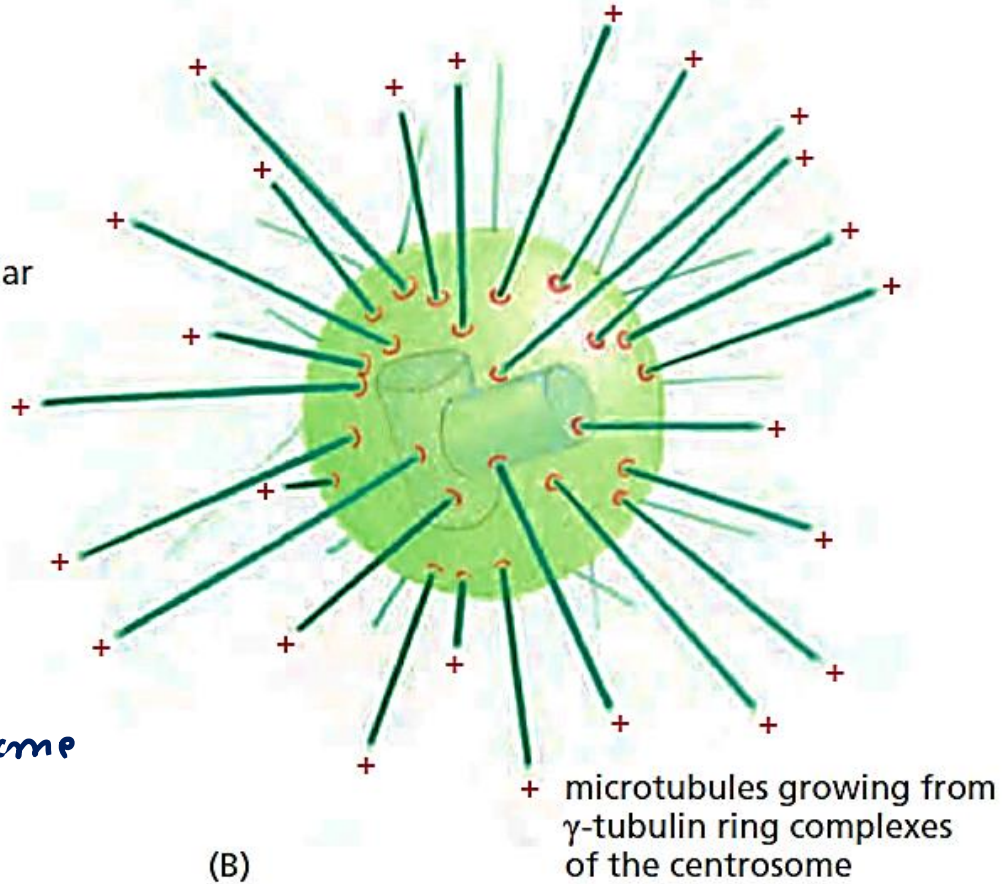
- Play role in karyokinesis cell elongation



Basal body (9 + 0)

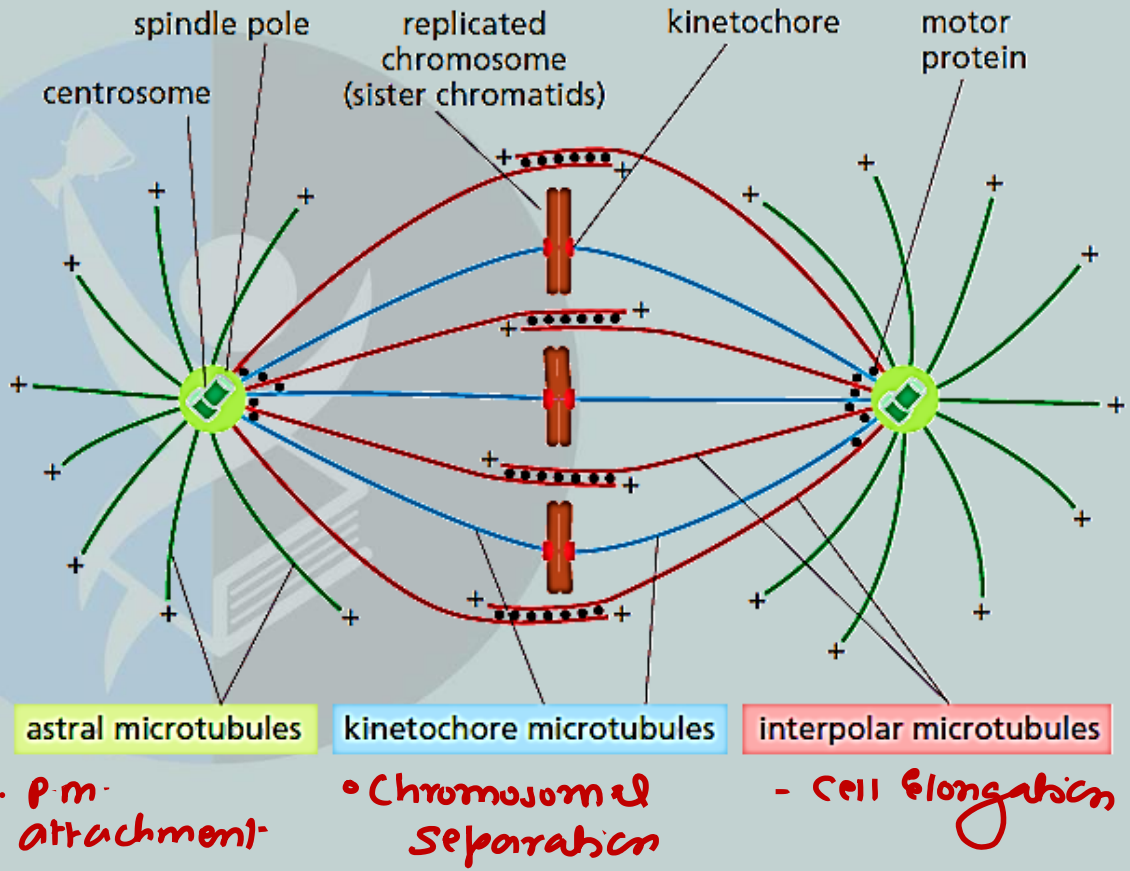
- form cilia and flagella


nucleating sites
(γ-tubulin ring complexes)
pericentriolar
material
pair of
centrioles
Centrosome
· fluid
No membrane
tnt
(A)

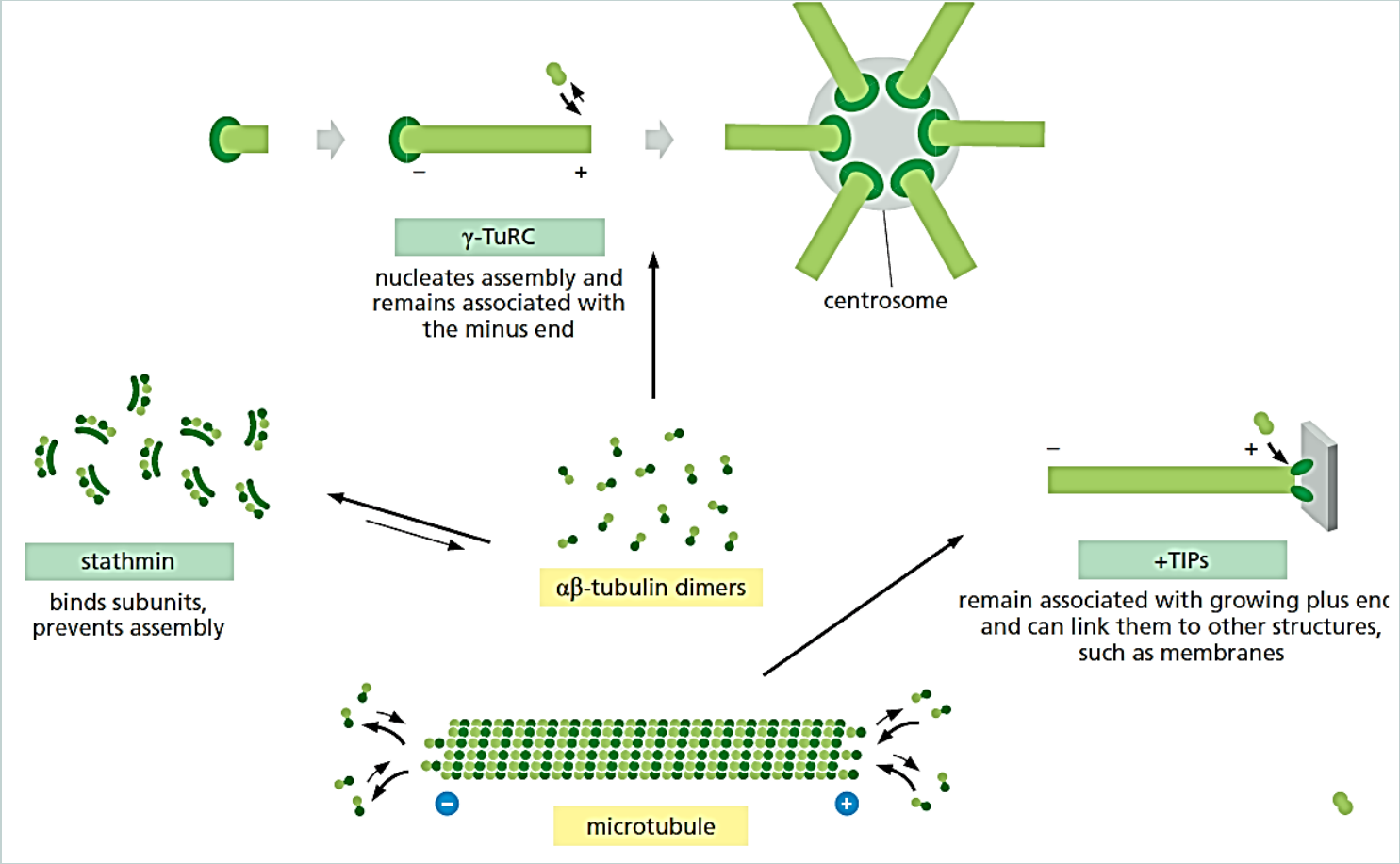

microtubules growing from
γ-tubulin ring complexes
of the centrosome
(B)

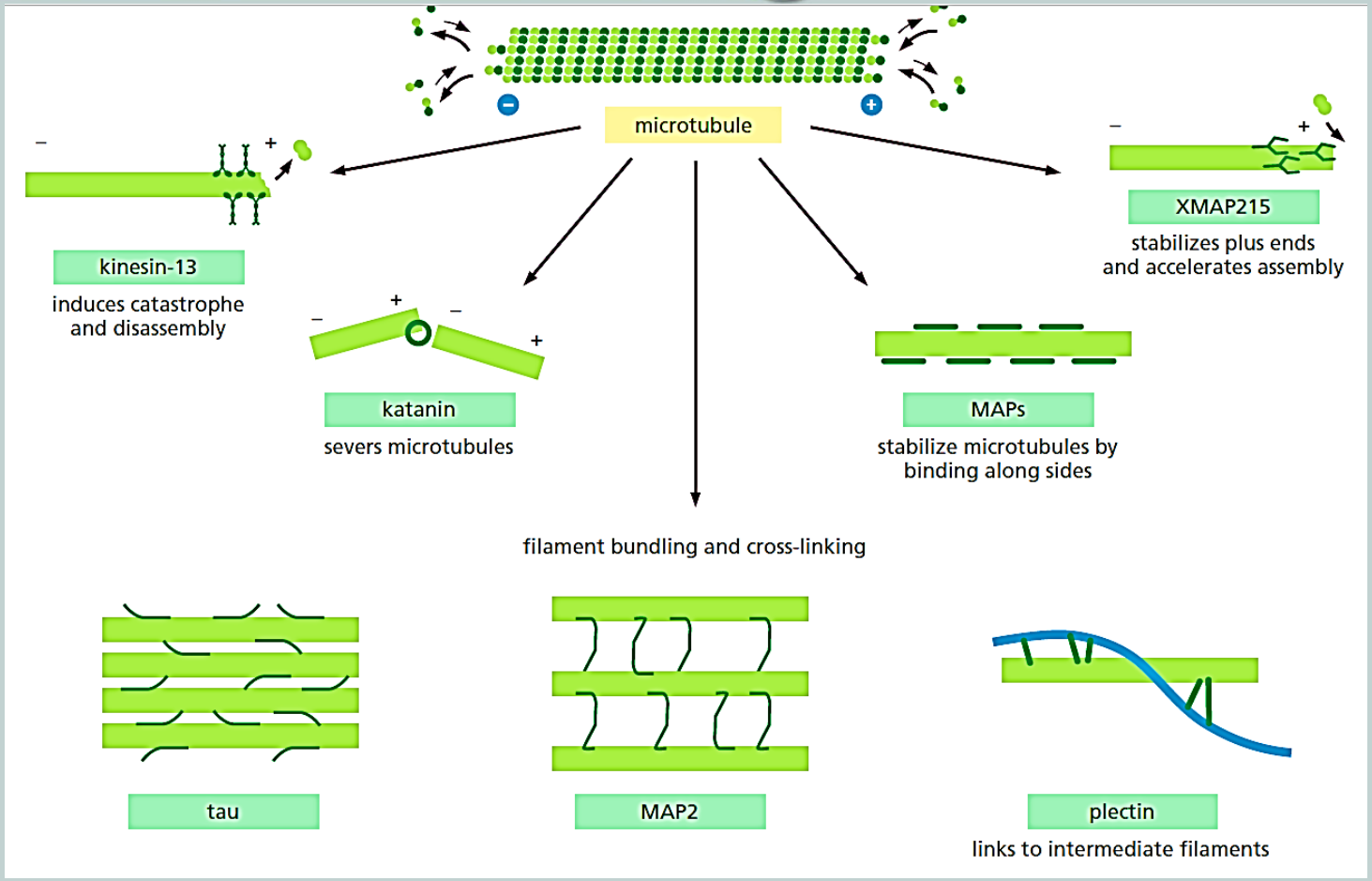
- Polar microtubule involve in cell elongation
- Chromosomal microtubule involve in arrangement of chromosome on metaphysic plane

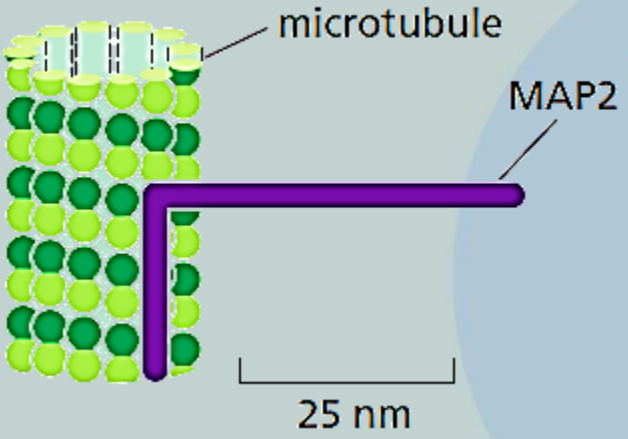
Hence astral microtubule attached with plasma membrane

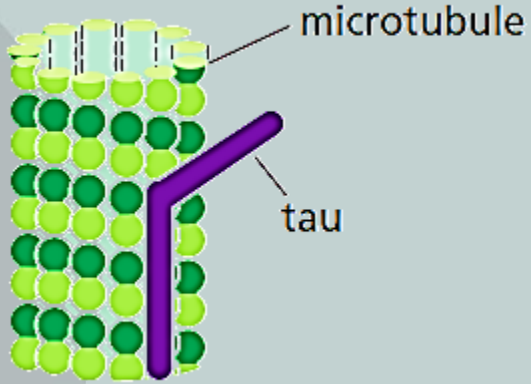


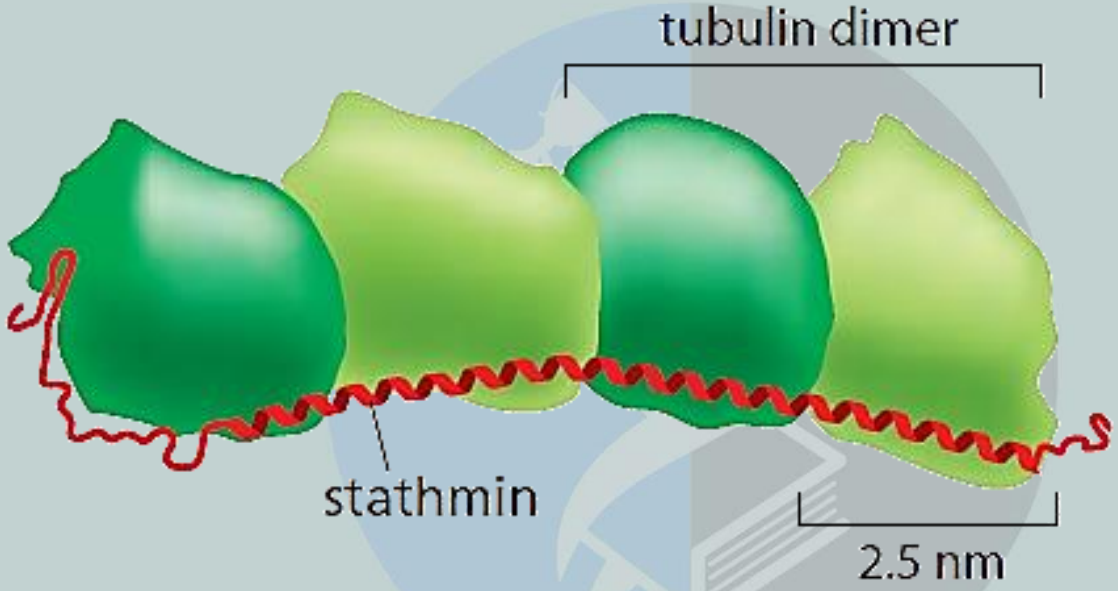
Protein that is associated with microtubule


γ-TuRC
nucleates assembly and remains associated with the minus end
centrosome
stathmin
binds subunits, prevents assembly
αβ-tubulin dimers
+TIPs
remain associated with growing plus end and can link them to other structures, such as membranes
microtubule

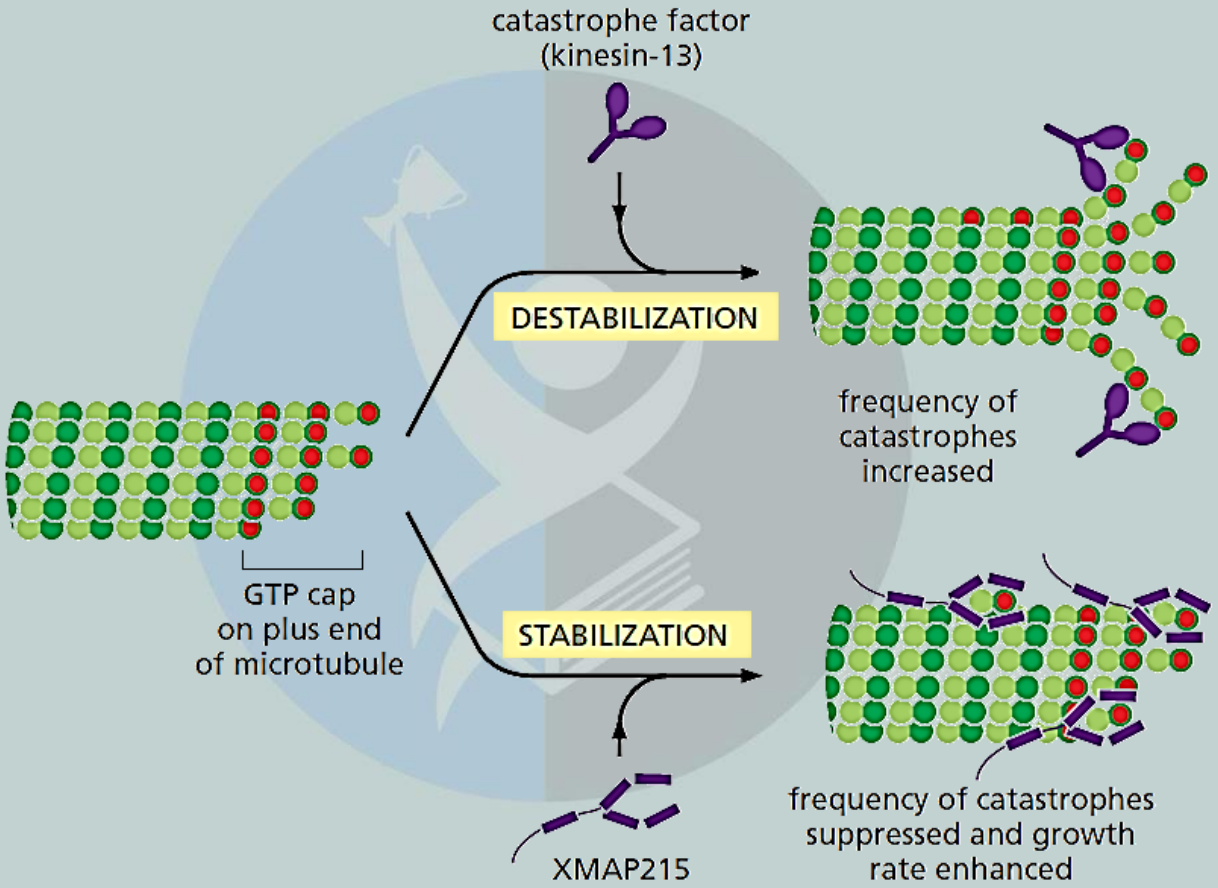

microtubule
kinesin-13
induces catastrophe and disassembly
XMAP215
stabilizes plus ends and accelerates assembly
katanin
severs microtubules
MAPs
stabilize microtubules by binding along sides
filament bundling and cross-linking
tau
MAP2
plectin
links to intermediate filaments


microtubule
MAP2
25 nm


microtubule
tau



Sequestration of tubulin by stathmin.


catastrophe factor
(kinesin-13)
DESTABILIZATION
frequency of
catastrophes
increased
GTP cap
on plus end
of microtubule
STABILIZATION
XMAP215
frequency of catastrophes
suppressed and growth
rate enhanced

Cellular Role	Representative proteins
Binds (+) ends and provides stability	+ TIPs
Microtubule severing	OP18/stathmin
Stabilize microtubules	MAP2 and Tau
Molecular Motors	Dynein moves cargo toward minus ends and activates sliding in flagella Classical Kinesins moves cargo toward plus ends of MTs

Molecular motor

work with actin filament

Myosin
- I → Chloroplast movement
- II → muscle contraction
- III
- IV
- V → Organelle movement

work with microtubule

Positive directed

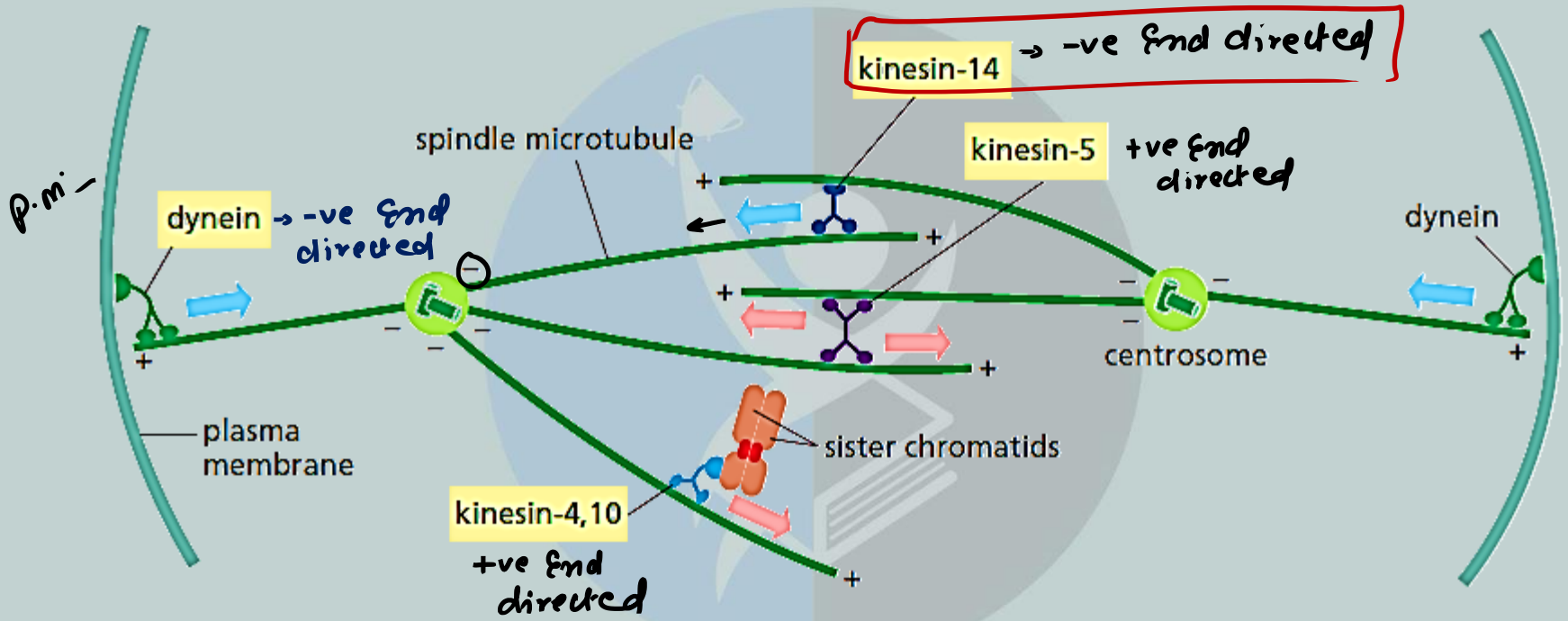
Kinesin 7 and 10

involve in vesicular transport Mostly from canter to periphery

- Anterograde path.

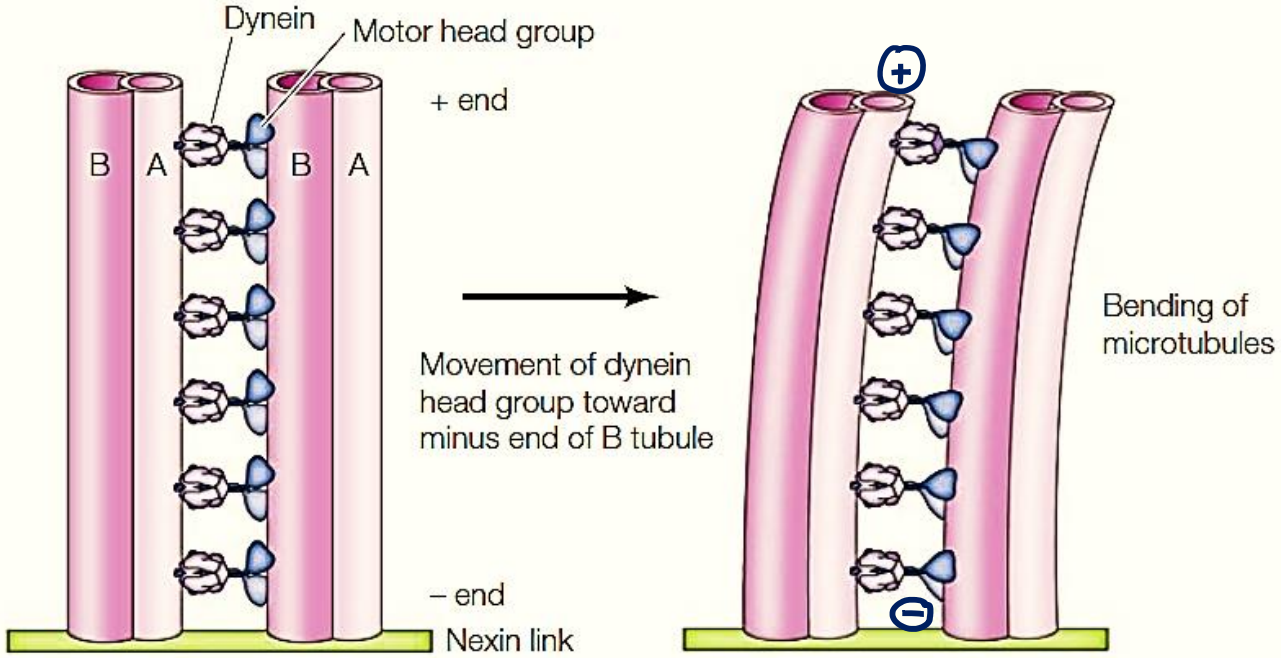
Negative directed

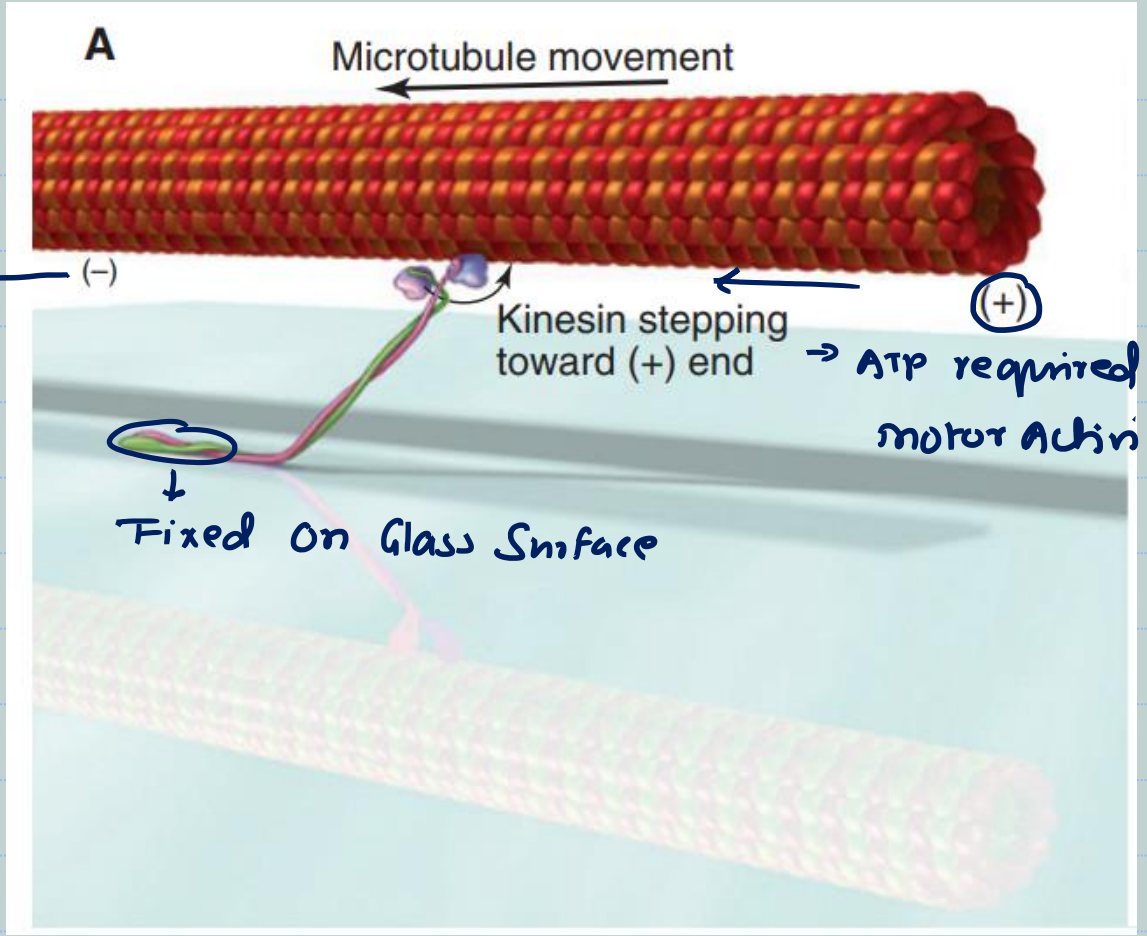
- Dynin
- Responsible for movement of cilia and flagella
- movement from Periphery to center (Retrograde path)

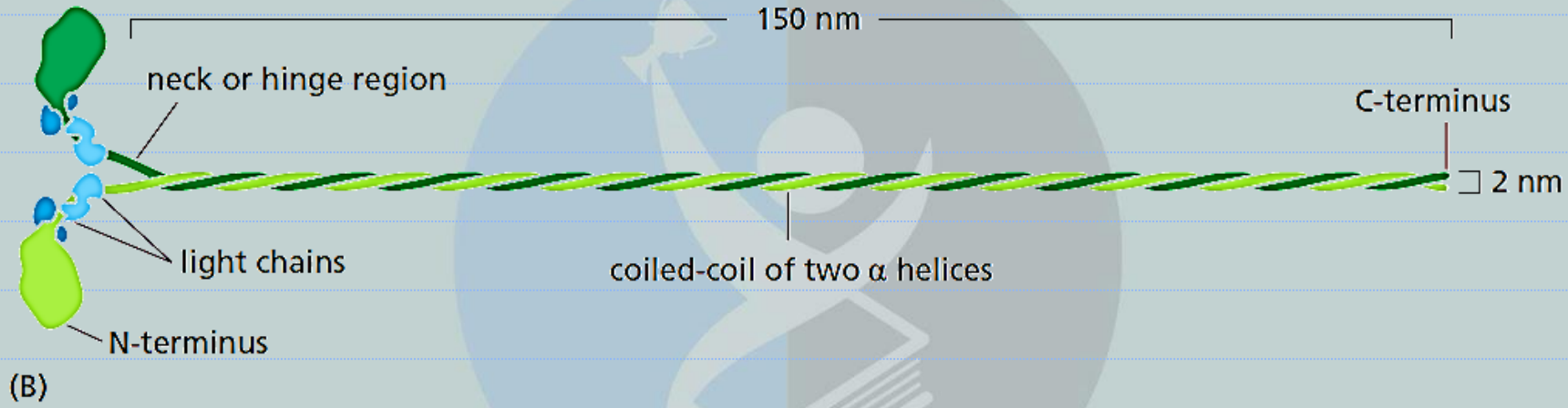

kinesin-14 → -ve end directed
spindle microtubule
kinesin-5 +ve end directed
dynein → -ve end directed
dynein
centrosome
plasma membrane
sister chromatids
kinesin-4,10
+ve end directed

Dynin negative directed motor

- Generate whip like motion




A
Microtubule movement
(–)
(+)
Kinesin stepping toward (+) end
→ ATP required for motor activity
Fixed on Glass Surface


150 nm
neck or hinge region
C-terminus
2 nm
light chains
coiled-coil of two α helices
N-terminus
(B)

Functions of microtubule

- ✓ Spindle fiber formation - Helps in chromosomal separation
- ✓ Vesicular transport (Anterograde (Kinesin), Retrograde pathway (Dynin))
- ✓ Organelle movement
- ✓ Movement of cellulose synthase

↓

during Plant cell wall Synthesis

Microtubule inhibitors are act as anticancerous drug

↓

Because these drug could inhibit karyokinesis And hence cell division

Drugs affecting microtubules

- Such drug will effect mitosis and thus cytokinesis will also be paused.
- **Colchicine** obtained from *Colchicum autumnale*, binds to tubulin monomers, inhibits assembly and promoting disassembly of existing ones. Induces polyploidy and arrest cell at metaphase.
- **Nocodazole** also inhibits microtubule assembly.
- **Vinblastine** and **vincristine** binds to the tubulin protein, stopping the tubulin dimers from polymerizing to form microtubules, causing the cell to be unable to separate its chromosomes during the metaphase. The cell then undergoes apoptosis

Chemical Inhibitors of Actin and Microtubules			
Chemical	Effect on filaments	Mechanism	Original source
Actin			
Latrunculin	Depolymerizes	Binds actin subunits	Sponges
Cytochalasin B	Depolymerizes	Caps filament plus ends	Fungi
Phalloidin	Stabilizes	Binds along filaments	*Amanita* mushroom
Microtubules			
Taxol® (paclitaxel)	Stabilizes	Binds along filaments	Yew tree
Nocodazole	Depolymerizes	Binds tubulin subunits	Synthetic
Colchicine	Depolymerizes	Caps filament ends	Autumn crocus

No. Polarity

Intermediate Filament - provide extra stability to the cell

Class	IF type	Tissue	Function
I	Acidic keratins	Epithelial cells	Mechanical strength
II	Basic keratins	Epithelial cells	Mechanical strength
III	Vimentin	Fibroblasts; lens of eye	Maintenance of cell shape
	Desmin	Smooth muscle cells	Structural support
	GFA protein	Glial cells and astrocytes	Maintenance of cell shape
IV	Neurofilament NL-F, M and H	Central and peripheral nerves	Axon strength; determines axon size
V	Nuclear lamins – A, B and C	All cell types	Form a nuclear scaffold
VI	Nestin	Neuronal stem cells	Unknown

Intermediate filament can be used as marker of origin of cancer cells.

Genetic disorder related to intermediate filaments include

- Epidermolysis bullosa simplex: Mutation in keratin gene
- Epidermolytic hyperkeratosis: Overexpression of keratin gene
- Hutchinson-Gilford progeria: Mutation in nuclear Lamin gene

Bacteria lack true cytoskeletal systems but have structurally similar components to those in eukaryotes

- the **actin-like** *MreB* protein is involved in DNA segregation,
- the **tubulin-like** *FtsZ* protein is involved in determining where bacterial cells will divide, and
- the **intermediate filament**-like *crescentin* protein is an important regulator of cell shape.

Cell wall

- present in bacteria, plants and fungi & -nt in Animal cell
- Present outside the plasma membrane
- Determines the shape and size of the cell

- Bacterial cell without cell wall can not maintain its shape = eg. Pleuro Pneumonia like organism [PPLO] (mycoplasma)

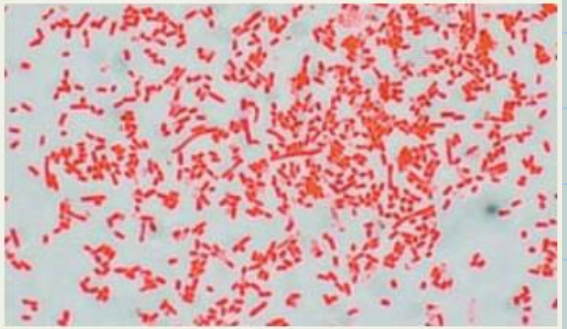
Eubacteria

Gram-positive

Stained with gram stain Crystal violet or gentian violet purple violet in color

Gram-negative

Loose gram stain after alcohol wash
Retain sepharanin i.e. counter-stain

Gram-Positive	Gram-Negative
	

Gram-positive bacteria

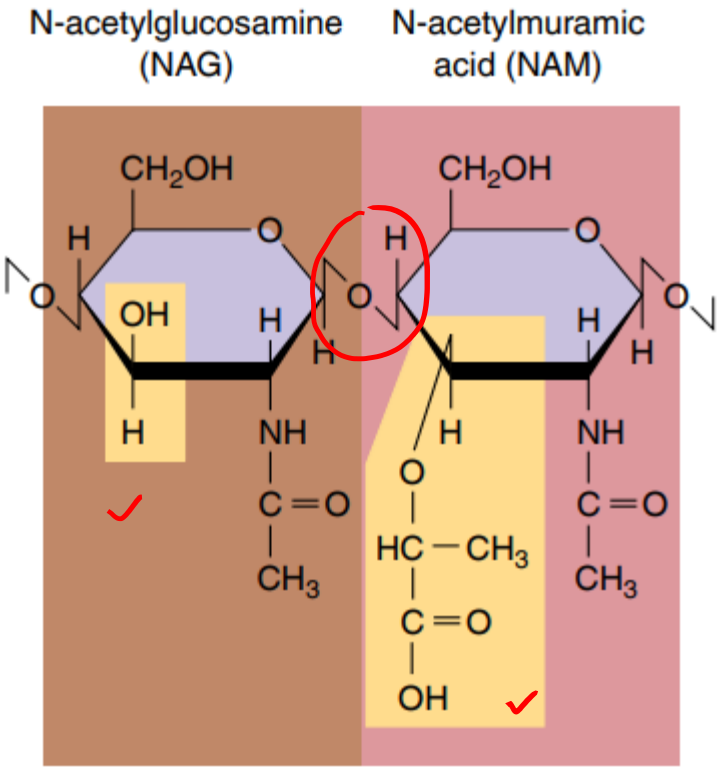
- Simple, structured, but thick cell wall

Cell wall composed of peptido-glycane

Peptido-glycane –

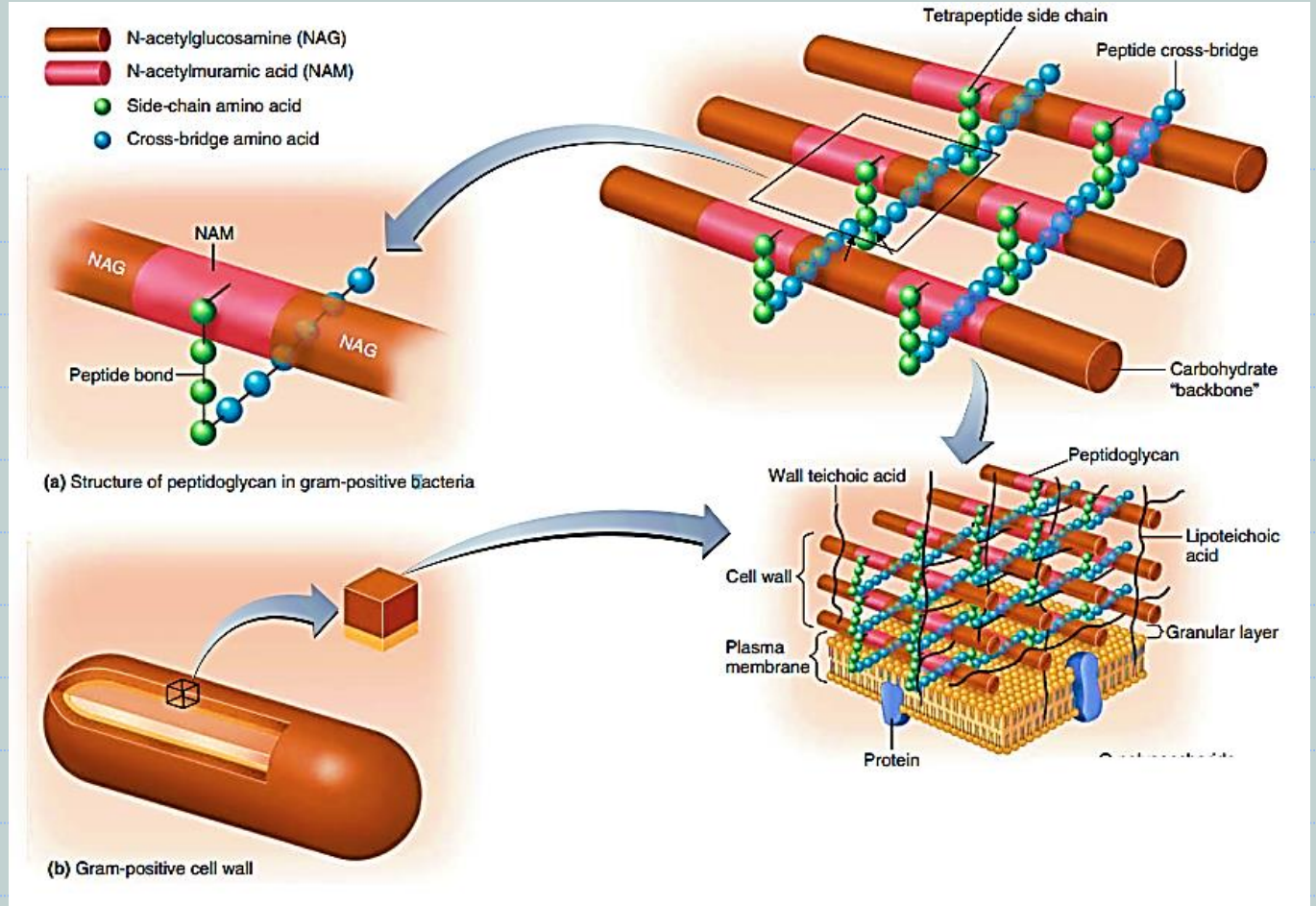
short peptide — large sugar

cross-linker of heteropolysaccharide Peptide chain contain D-L and [illegible] form of amino acid

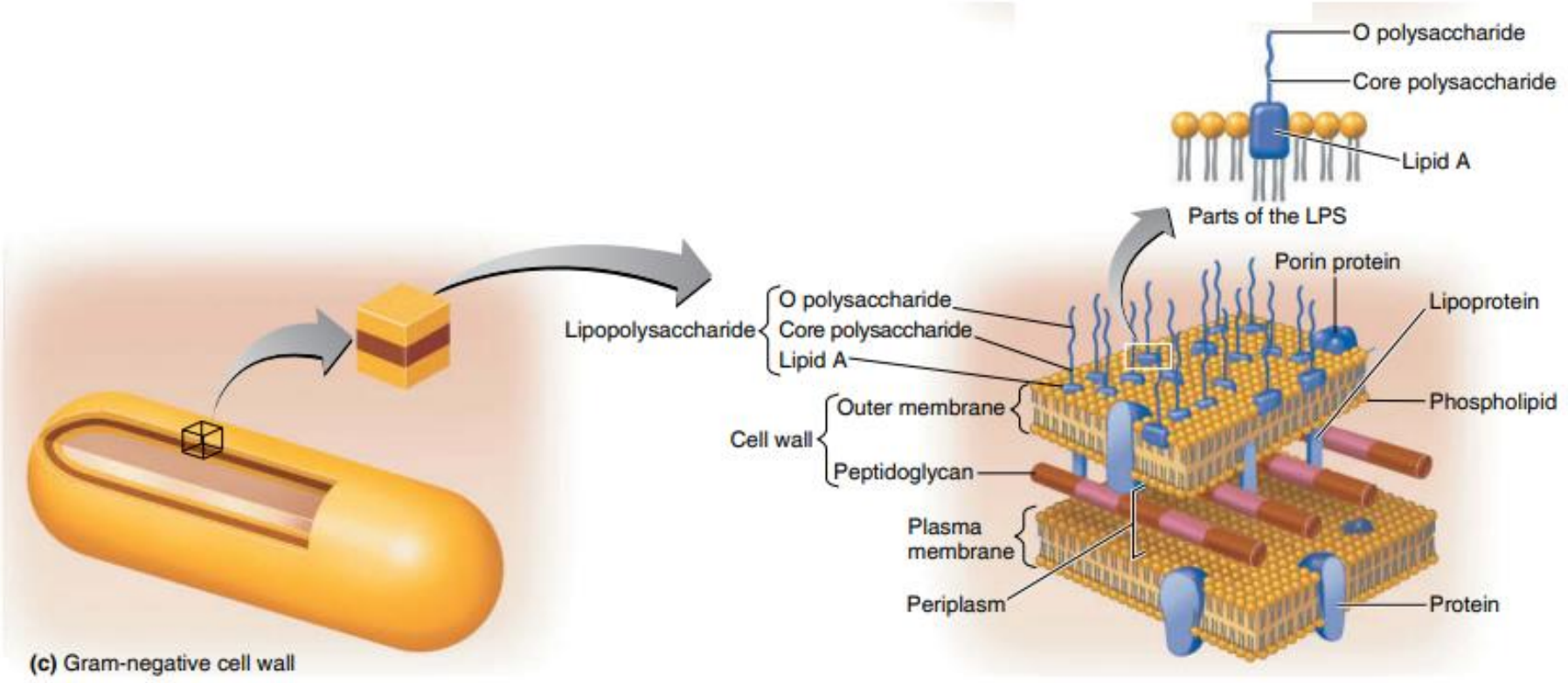


- Large sugar Heteropolysaccharide
- (1) N-acetylglucosamine (NAG)
- (2) N-acetylmuramic acid (NAM)

  Both sugar attached with Beta-1,4-glycosidic linkage


N-acetylglucosamine (NAG)
N-acetylmuramic acid (NAM)
Side-chain amino acid
Cross-bridge amino acid
Tetrapeptide side chain
Peptide cross-bridge
NAM
NAG
NAG
Peptide bond
Carbohydrate "backbone"
(a) Structure of peptidoglycan in gram-positive bacteria
Peptidoglycan
Wall teichoic acid
Lipoteichoic acid
Cell wall
Granular layer
Plasma membrane
Protein
(b) Gram-positive cell wall

Techoic acid provide extra stability Peptide cross-linker


O polysaccharide
Core polysaccharide
Lipid A
Parts of the LPS
Porin protein
Lipoprotein
Lipopolysaccharide
O polysaccharide
Core polysaccharide
Lipid A
Phospholipid
Cell wall
Outer membrane
Peptidoglycan
Plasma membrane
Periplasm
Protein
(c) Gram-negative cell wall

Gram-negative bacteria

E.g. E.coli

Thin layer of peptido-glycane

Lipopolysaccharide present on outer membrane of cell wall

Lipo-represent lipid 5%

Lipopolysaccharide act as antigen that is present on outer membrane of gram-negative bacteria

Polysaccharide 95% part Lipopolysaccharide contain O-antigen Disaccharide Lipid-A act as strong endotoxin → Part of cell wall

Lysozyme inhibit beta-1,4-glycosidic linkage (Bacterial cell wall)

Penicillin and endopeptidase inhibit peptide cross-linking

(Beta-lactam- penicillin) inhibit peptide cross-linking

* Peptide bridge contain L-alanine (1) D-glucose (2) D-alanine (3) Alpha-diaminopimellic acid/L-lysine present in gram-positive bacteria (4)
Diaminopimellic acid most common in gram-negative bacteria

DAP / L-Lys

↓ Gram -ve

↓ Gram +ve

Archaeobacteria

- present with ether bond in Fatty acyl chain
- Plasma membrane contain tetra-ether
- Cell wall peptido-glycane absent
- Pseudomurine present So cell wall is made up of psudo -peptido-glycane

* Psudomurine Present insted of NAM.

Fungal cell wall made up of chitin

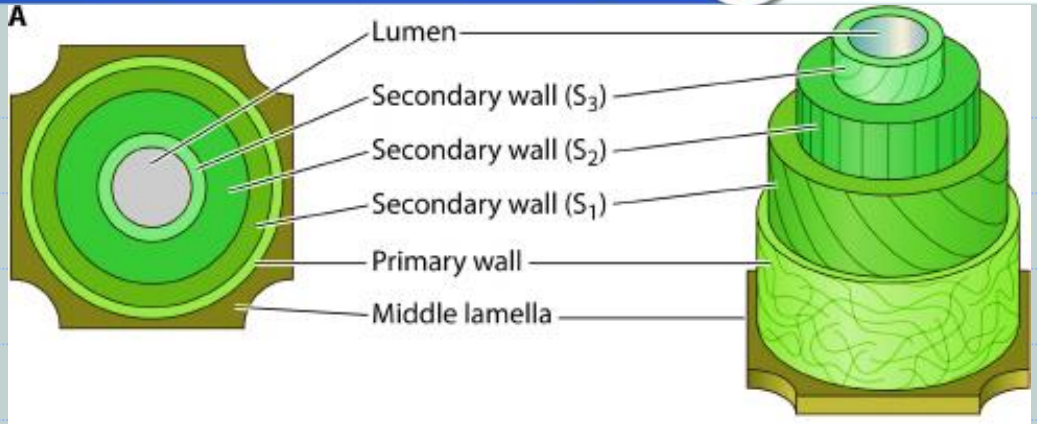
Exception O-mycetes Cell wall composed of cellulose

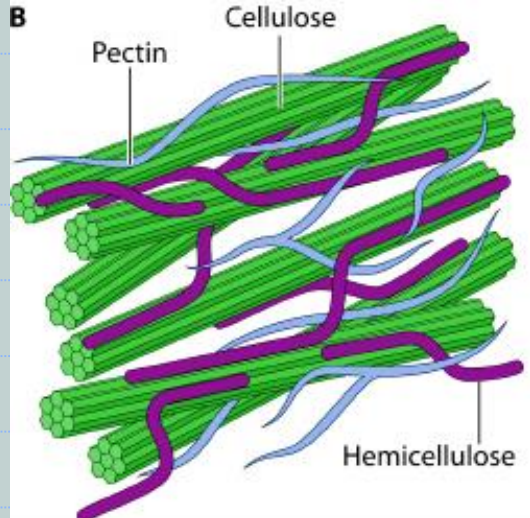
Plant cell wall - Cellulose is the main component

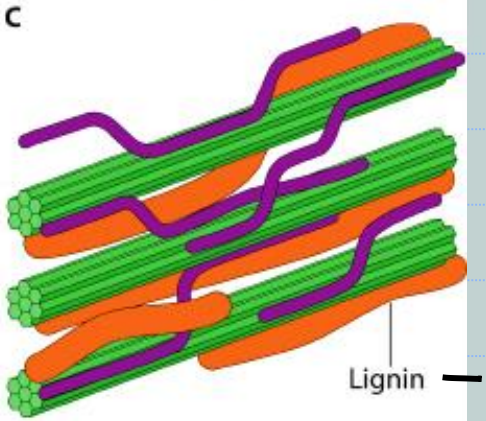
$(Glu)_n$ = cellulose + Hemicellulose] plant cell wall

Cellulose Homopolymer cross-linked with hemicellulose

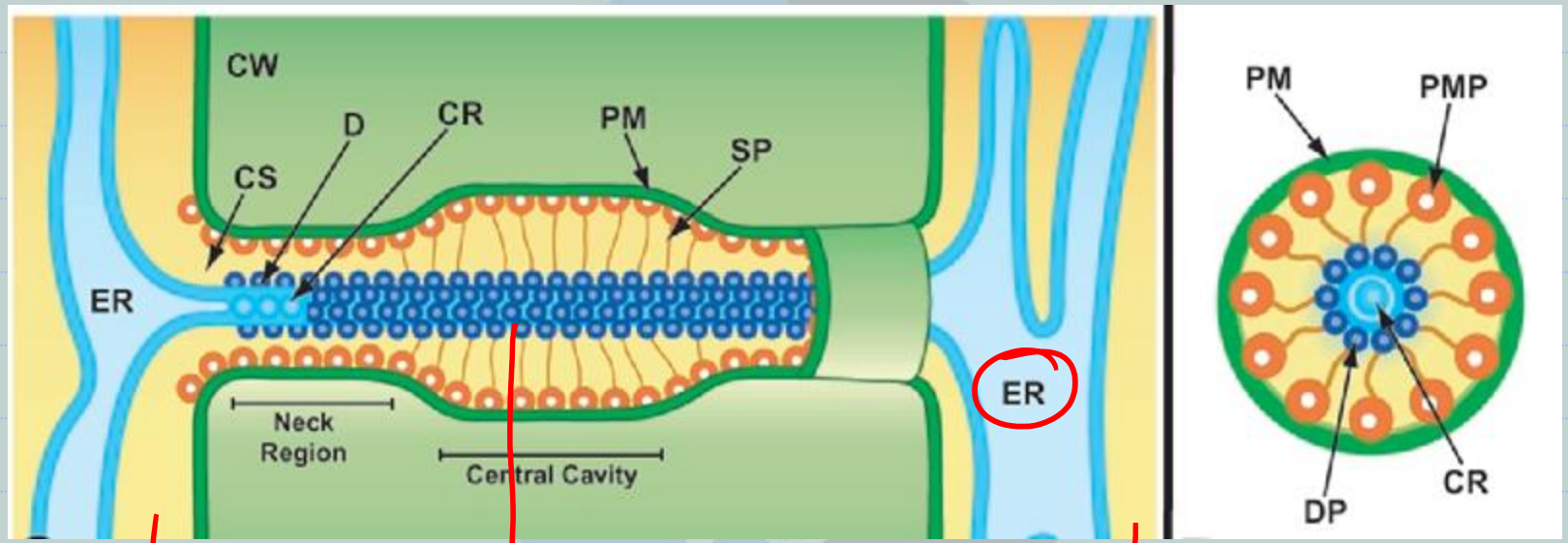
- Secondary cell wall contain Cellulose, hemicellulose, lignin, woody brown substance
- Primary cell wall contain cellulose, hemicellulose, pectin Plasmodesmata act as gap junction







Brown woody Substance

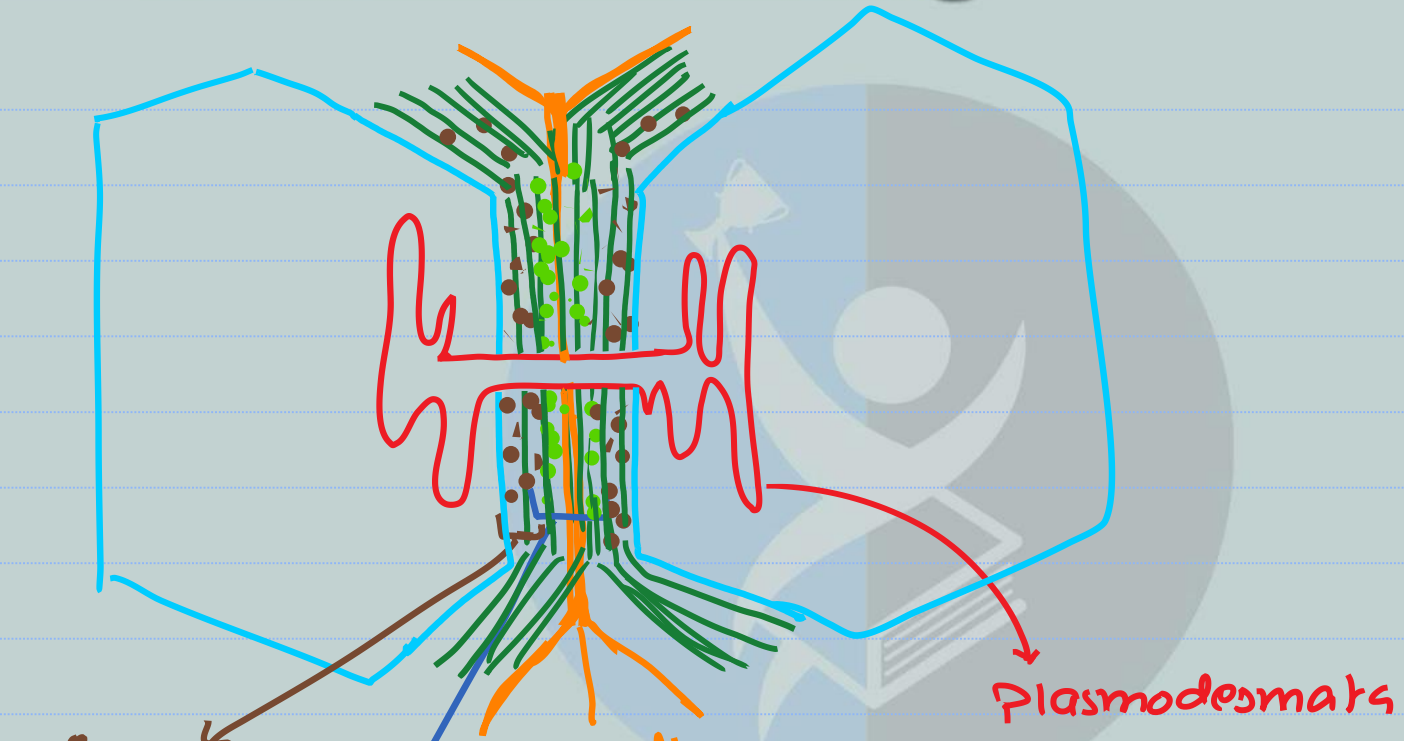


cell

Plasmodesmata

Gap junction of plant cell

cell


Plasmodesmata
2° cell wall
middle lamilla [Pectin]
Primary cell wall

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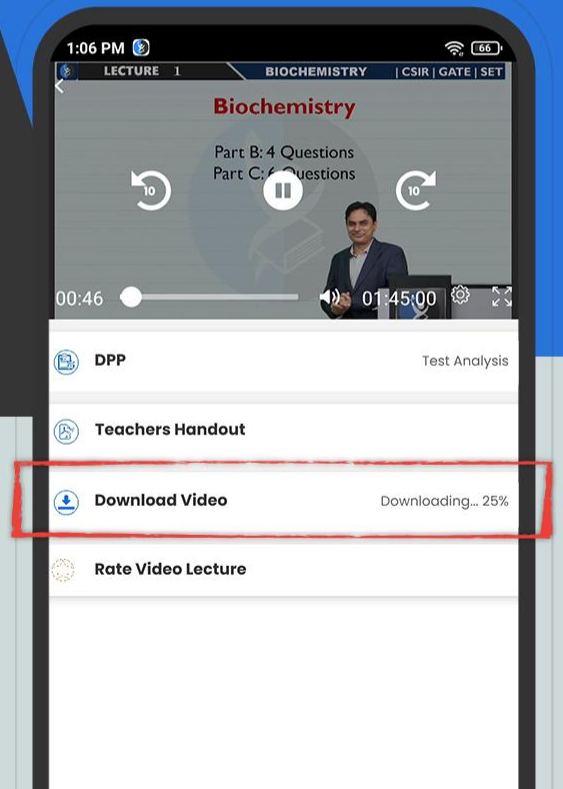

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